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A Dissertation on

SEROPREVALENCE OF HUMAN CYTOMEGALOVIRUS AMONG VOLUNTARY BLOOD DONORS IN CHENNAI

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

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INTRODUCTION

Blood transfusion is a life saving modality. The transfusion of blood and blood products is much safer than ever before but still a long way from achieving universal access to safe blood transfusion.[1]

A *Transfusion Transmitted Infection* (TTI) is any potential pathogen that can be transmitted in donated blood through a transfusion to a recipient. The magnitude of the problem of transfusion transmitted diseases varies from country to country depending on disease prevalence. Various measures are taken in a country to make blood transfusion therapy safe for the respective population.

There is a long list of viruses, parasites and bacteria and recently prion diseases, which can be transmitted through blood transfusion. Majority of the problems are due to the prevalence of asymptomatic carriers in the society as well as blood donations during window period of infections. Viral infections assume a great importance in transfusion associated mortality and morbidity in patients. Important transfusion transmitted viruses are HIV, HBV, HCV, HTLV, Parvo virus B-19 and cytomegalovirus.[2]

Over the years, there has been a substantial decline in the incidence of transfusion-transmitted infections due to improvement in donor screening, blood product testing and viral inactivation of blood products, particularly in developed nations. However, in developing nations, blood safety continues to be a major problem due to the high prevalence of infectious markers among blood donors compounded with the problem of limited resources that preclude the use of sophisticated, sensitive but expensive technologies for screening of blood products.[3]

The last two decades have also witnessed surfacing of new and re-emerging infections. Hence, despite stringent donor eligibility criteria, improved donor screening and introduction of sophisticated technology, transfusion-transmitted infection continues as a challenge for transfusion experts.

Cytomegalovirus (CMV), a member of the human herpes family of viruses, transmissible through blood transfusions, is an important cause of concern world wide.[4]

CMV is a ubiquitous agent and seropositivity rates in the population are 60 to 100%.[5] CMV is one of the most significant pathogens infecting immuno suppressed individuals. Like most other herpes viruses, they remain latent in the host after primary infection and

persist for life long in the organism. Nevertheless, these viruses can be reactivated in immuno suppressed individuals leading to various critical outcomes.[6]

In immunocompromised recipients, transmission rates of up to 50% have been reported from blood components.[7]

Therefore, the most effective way to minimize the risk of CMV transmission in high risk recipients would be to administer CMV free blood products. The immuno suppressed population for whom CMV free blood products are requested is increasing due to advances in medical care. This means that considerable stress is placed on blood banks to maintain adequate inventory of these products.[8]

In view of the increasing demand for CMV free blood products, this study was performed to determine the seroprevalence of CMV antibodies among voluntary blood donors.

An estimate of the seroprevalence of CMV among voluntary blood donors may be of help to decide whether screening for CMV would eliminate transmission of infection to high risk groups. Such studies have been very few in India. The current study was undertaken in an attempt to address this aspect. Such information may be invaluable to health planners and policy makers.

AIM AND OBJECTIVES

AIM

The aim of the study is to find out the seroprevalence of Human Cytomegalovirus among voluntary blood donors in Chennai.

OBJECTIVES

- To detect IgM and IgG anti-CMV antibodies among healthy blood donors.
- To confirm CMV seronegative samples for CMV DNA by PCR analysis.

REVIEW OF LITERATURE

Blood transfusion is an essential element of a health care system. Millions of lives are saved each year through blood transfusions. Inadequacies in blood safety and supply, contribute significantly to the burden of disease and loss of life.[9]

Blood transfusion has revolutionized modern medicine. By maintaining blood volume, replacing deficient blood components and improving oxygen transportation, transfusion has expanded the boundaries of modern medicine, allowing many crucial surgical procedures, organ transplants, and cancer therapies to be performed.

Blood transfusions, however, are not risk-free. Despite significant improvements in safety measures, blood transfusions are still associated with a residual risk of infection by various pathogens, many of which are serious and life threatening. The safety assessment of the blood supply, the quality of screening procedures, and the risk of transfusion-transmitted infectious diseases in any country can be estimated by review and analysis of the records of blood donors, screening procedures, and the prevalence of serological markers of infectious diseases.[10]

A report of an infection suspected to be due to transfusion was classified as a transfusion-transmitted infection if the following criteria were met at the end of the investigation.[11]

a) The recipient had evidence of infection post-transfusion, and there was no evidence of infection prior to transfusion and no evidence of an alternative source of infection

b) Atleast one component received by the infected recipient was donated by a donor who had evidence of the same transmissible infection, or

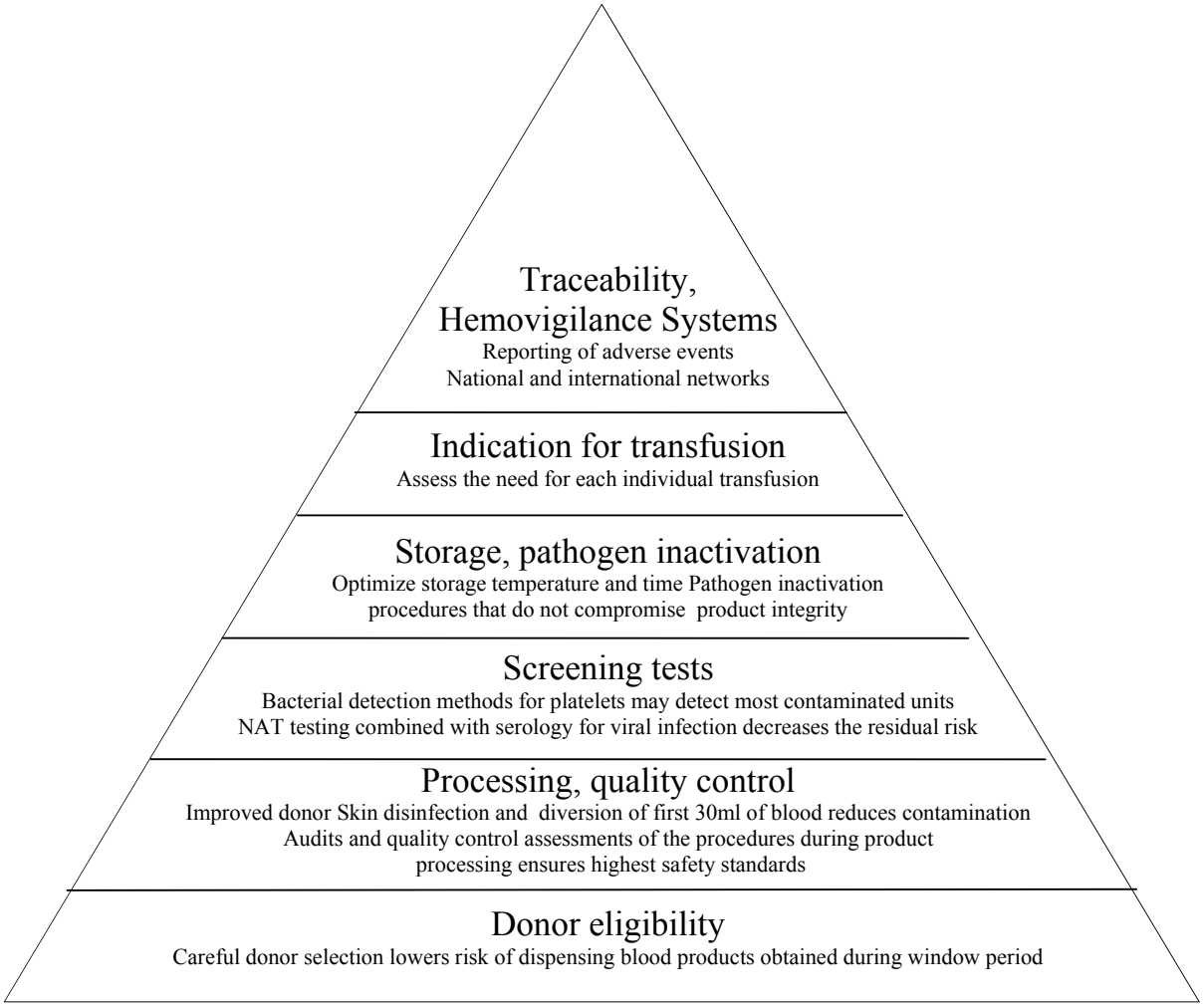
c) Atleast one component received by the infected recipient was shown to contain the agent of infection.

The goal of any transfusion service is to provide blood components that are safe for the transfusion and pose minimal risk of transfusion transmitted infections.

Transfusion transmissible agents have certain characteristics.[12]

1. Persistence in blood for relatively long period of time
2. Giving rise to carrier or latent state
3. Causation of disease with long incubation period
4. Ability to cause symptomatic infections
5. Stability in cold stored blood

To achieve maximum safety at an acceptable cost, it requires a multilayered risk reduction strategy involving safe blood donors, safe blood components and safe transfusion practices.[13]



Multilayered risk reduction strategy[13]

Blood transfusion is a unique technology that blends science with altruism. Though its collection, processing and use are technical, its availability depends entirely on the extraordinary generosity of the blood donor who donates this most precious of gifts – the gift of life. Safe transfusion not only requires the application of science and technology to blood processing and testing, but also social mobilization to promote voluntary blood donation by sufficient numbers of people who are healthy and are at low risk of infections that can be transmitted to the recipients of their blood.[14]

Voluntary Blood Donors form the cornerstone of a safe and adequate supply of blood and blood products. Voluntary blood donor refers to unpaid, non-remunerated blood donors. He is an altruistic donor who gives blood freely and willingly without receiving money or any other form of payment.[15]

Over the past decade, efforts have been made to quantify the risks of transfusion-transmitted infectious diseases accurately. Although, numerous analyses on risk of human immunodeficiency virus (HIV) infection have been made, there are fewer reliable estimates of infection rates for the other major transmissible agents by transfusion. Accurate estimations on risk of transfusion transmitted viral infections are needed, in order to monitor the safety of the blood supply and evaluate the cost effectiveness of new screening tests.

The most direct way to evaluate the transfusion associated risk is to study the rate of infection prospectively in transfusion recipients. The current very low risk of transfusion transmitted infectious diseases makes such studies impractical because an exceedingly large number of recipients are required for the risk to be measured accurately. Alternatively, the infection rate in donated blood samples can be determined by testing with extremely sensitive assays.[10]

In India, donor blood is screened for Human Immunodeficiency Virus I & II (anti-HIV), Hepatitis B virus (HbsAg), Hepatitis C Virus (anti-HCV), syphilis and malaria.[16]

HUMAN HERPES VIRUS

The Herpesviridae is a family of approximately 100 viruses with common structural features. Of the herpes viruses, only eight are known to infect human beings, which are termed as ***Human Herpes Viruses***. Members of the Human Herpes Virus (HHV) family are categorized into three subfamilies based on biologic properties including cell tropism, genome structure and sequences of conserved open reading frames.[17]

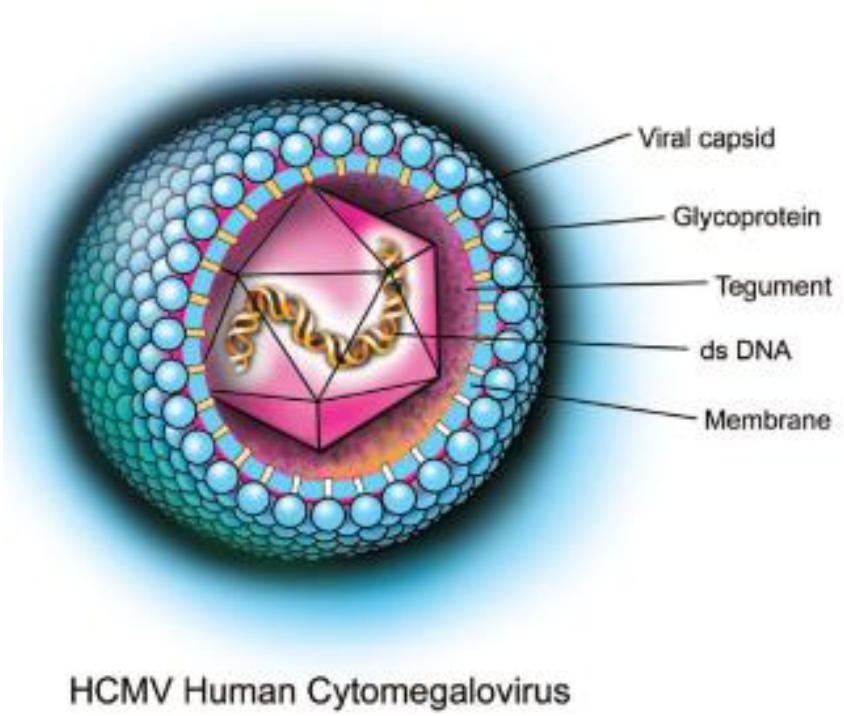
Members of the Herpesviridae Family[18]

SUBFAMILY	HHV DESIGNATION	COMMON NAME
Alphaherpesvirinae	HHV-1	Herpes simplex-1
	HHV-2	Herpes simplex-2
	HHV-3	Varicella Zoster Virus
Betaherpesvirinae	HHV-5	Cytomegalovirus
	HHV-6A,-6B	-
	HHV-7	-
Gammaherpesvirinae	HHV-4	Epstein-Barr Virus
	HHV-8	Kaposi's sarcoma associated Herpes virus

CYTOMEGALOVIRUS

Cytomegalovirus (from the Greek *cyto-*, "cell", and *-megalo-*, "large") is a human herpes virus belonging to the Betaherpesvirinae subfamily. It is designated as *Human Herpesvirus 5* (HHV-5). CMV was the first identified beta herpes virus and remains the prototype of this group.[19]

STRUCTURE



HCMV Human Cytomegalovirus

The CMV virus contains a linear double-stranded DNA genome of approximately 230 kbp in length, the largest of the herpes virus. The genome is surrounded by an icosadeltahedral nucleocapsid. The 100 nm diameter capsid, composed of 162 capsomeres, is encompassed by a dense tegument or matrix and an outer trilaminar lipid envelope that contains proteins of both viral & host cell origins.[20]

The genome is divided into unique long (UL) and unique short (US) segments, each flanked by a pair of inverted repeat regions.[21] The unique long and unique short segments can each independently invert with respect to one another, yielding four different genomic isomers.

After infection, the termini of the linear genome are joined to produce a circular replicative form. Mature virions range from 150-200 nm in diameter and contain approximately 30 viral proteins distributed in the capsid, tegument and envelope.[22]

BIOLOGY OF INFECTION

CMV can infect a range of cell types, including those of endocrinal, epithelial, mesenchymal, hematopoietic and neuronal lineages; frequently causing characteristic cellular enlargement-cytomegalia (Fig 1).[23] Infections appear to involve three sequential steps:

1. Viral attachment to the target cell.
2. Fusion of the viral and cellular membranes.
3. Penetration of the viral capsid into the cell.

After these steps, active infection occurs if the target cell is permissive for the complete sequence of viral gene expression, viral genome replication and production of progeny virions. Finally mature virions are transported through the Golgi apparatus and are released from infected cells by exocytosis eventually resulting in host cell destruction.[24]

CMV may also assume a latent state when it infects target cells that are not permissive for viral replication. Latency, the presence of viral DNA in an infected cell in the absence of active viral replication, may persist indefinitely because the host cell is not destroyed by the virus. The latent CMV genome retains the capacity to reactivate viral gene expression, produce infectious virions, and enter lytic growth at a later time.[25]

CMV infection alters the expression, accumulation and activity of the cellular tumour suppressor proteins, cyclins and cyclin associated kinases. These alterations in the cell cycle machinery act to promote progression toward the G1/S transition but prevent cellular DNA synthesis and cell division, resulting in cell cycle arrest and cellular aneuploidy. It has been hypothesized that in the arrested state, cellular DNA synthesis would be blocked but the cellular milieu would contain abundant nucleotides and other metabolic processors that could support viral replication.[26, 27]

Slobedman et al demonstrated that latency can be established in hematopoietic cells, primarily those of the Granulocyte-Monocyte lineage.[28] Monocytes are a prominent site of CMV latency and monocyte derived macrophages can support active CMV replication.[29]

CMV TRANSMISSION

During CMV infection, active viral replication results in shedding of infectious virions into plasma and body fluids including saliva, tears, breast milk, urine, stool and semen.

Community acquired CMV infection is usually the result of close contact with a person shedding CMV.

The yearly CMV seroconversion rate in health care workers has been estimated at 0.6 to 3.3%, similar to rates of 2% to 6.3% reported in middle class women during and between pregnancies.[30]

In contrast, rates as high as 13% per year have been observed in adolescents.[31] The CMV seroconversion rate in blood donors is estimated at approximately 1% per year.[32, 33]

Most individuals contracting community acquired CMV infection are immunocompetent and the infection is often asymptomatic. However, a mild self-limited infectious mononucleosis syndrome can occur, with symptoms that include fever, malaise, hepatosplenomegaly and a rash. CMV can be isolated from body secretions during the symptomatic phase. The infected individual mounts both a humoral and cell mediated immune response and viral symptoms rapidly resolve, leading to a complete recovery. However, despite effective control of CMV infection by the competent host immune system, the virus is not completely eliminated but instead becomes latent.

Presentations of Acute Cytomegalovirus Infection in a Normal Person[34]

Common	Less common	Rare
Asymptomatic (most common)	Exudative pharyngitis	Icteric hepatitis
Mononucleosis syndrome	Splenomegaly	Guillain-Barré syndrome
Fever	Cervical adenopathy	Encephalitis
Malaise	Nonspecific rash	Myocarditis
Sore throat	Anemia	Pneumonitis
Headache		
Increased levels on liver function tests		
Lymphocytosis		
Antibiotic rash		

CMV can be transmitted by blood transfusion, transplacental route or by transplantation of hematopoietic stem cells and solid organs from infected donors. When the recipients are immunocompromised, CMV transmission through these mechanisms can produce serious clinical consequences.

PATHOGENESIS

The most important target cells of CMV infection are peripheral blood leukocytes and their progenitors. These cell types can either harbor latent CMV or allow active viral replication and thus are well suited to mediate transfusion transmitted CMV infection.

CMV infection of bone marrow hematopoietic progenitor cells (CD34+ cells) likely occurs during primary infection. Most evidence suggests that these cells restrict viral replication but support viral latency, although some studies have shown low levels of CMV replication.[35] Because of their capacity for self renewal, latently infected hematopoietic progenitor cells represent a potential long term reservoir of latent virus. Latently infected marrow progenitor cells are a likely vector for transmission of CMV infection by hematopoietic stem cell transplantation.

Myeloid lineage committed CD33+ progenitor cells also appear to be latently infected. As CD33+ progenitors continue to differentiate, they enter the peripheral blood. Monocytes appear to retain latent virus, but as they differentiate into macrophages, CMV replication with production of progeny virus has been observed. These findings support a model for latency in which early hematopoietic progenitor cells are latently infected during primary infection and thereafter serve as viral reservoirs.

Tolpin et al provided the first biochemical and molecular evidence for transfusion transmitted CMV infection. They reported that cells of the monocyte lineage have been hypothesized to mediate transfusion transmitted CMV, but the prevalence of latently infected monocytes in the peripheral blood appears to be low.[36]

Kenneth et al indicated that WBCs of the monocyte lineage are the most likely to carry latent CMV in seropositive donors.[35] Circulating latently infected monocytes must be able to support viral reactivation from latency to mediate transfusion transmitted CMV. These cells became permissive for CMV reactivation and viral replication after exposure to T-Cell conditioned medium and hydrocortisone or the combination of IFN-2, TNF and IL-4 respectively.[37]

It has been estimated that 0.01% to 0.12% of Peripheral blood mononuclear cells (PBMNC) from healthy seropositive blood donors contain CMV DNA with a range of 2 to 13 viral genomes per infected cell.[28] Approximately 5% of PBMNC are monocytes, so latently infected monocytes may comprise only 1 to 25 of every million peripheral blood WBCs. This low number of latently infected leukocytes in transfused blood components may contribute to the variable incidence of transfusion transmitted CMV observed clinically.

Plasma free virus appears to be less stable than intracellular virus and the presence of free virus in plasma is usually transient.[38] Zanghellini et al reported that in recently infected adolescents, 25-40% had plasma viremia, which was rarely identified more than four months after seroconversion.[31]

TRANSFUSION TRANSMITTED CMV INFECTIONS

By the mid 1960s, a number of investigators had described an illness with clinical similarities to infectious mononucleosis occurring in patients who were exposed to blood products during cardiopulmonary bypass for open heart surgery.[39] Patients typically presented with fever, splenomegaly and atypical lymphocytosis within 3-8 weeks of surgery but had a negative heterophile antibody test and did not experience exudative pharyngitis or lymphadenopathy.

Klemola et al and kaariainen et al subsequently demonstrated an increase in the titer of complement fixing anti-CMV antibodies concurrent with the illness, suggesting that the etiology was CMV infection acquired from transfused blood products.[40, 41]

Transfusion can lead to active CMV infection in the recipient by three mechanisms.

I) The term **Transfusion Transmitted CMV** (TT-CMV) is used to describe a primary CMV infection occurring in a seronegative recipient transfused with an infectious blood component.

II) **Reactivated CMV** infection can occur when a seropositive transfusion recipient experiences reactivation of their latent CMV infection after blood transfusion.

III) **CMV super infection** (second strain infection) occurs when a seropositive recipient contracts a new strain of CMV from an infectious blood component.

Transfusion transmitted CMV results in a primary infection against which the recipient has no pre existing immunologic memory. In contrast, CMV reactivation and super infection occurs in a patient having previous CMV infection.

The diagnosis of both reactivation and super infection is based on a four fold or greater rise in the titer of anti CMV antibodies and/or renewed viral shedding in secretions of seropositive transfusion recipients.[42] The incidence of CMV reactivation is independent of donor serostatus.[43] Although reactivation and super infection can be distinguished from one another by restriction endonuclease genotyping of CMV strains, this analysis has no significant clinical implications.[36]

These three mechanisms of transfusion associated CMV infection appear to occur with similar frequencies. A review by Adler et al, about transfused CMV seropositive patients, calculated a 26% cumulative incidence of CMV reactivation or super infection compared to a 31% incidence of transfusion transmitted CMV (primary CMV infection) in seronegative recipients.[42]

It should be noted that although most cases of suspected transfusion transmitted CMV result from the transfused component, a minority of cases may result from community-acquired CMV infection.[44]

BLOOD COMPONENTS ASSOCIATED IN TT-CMV

The primary vector for TT-CMV is the CMV infected leukocyte. All cellular components are implicated in transmitting CMV infection. TT-CMV has not been observed in patients receiving blood components that are free of WBCs. The absence of CMV transmission through transfusion of FFP may be due to the scarcity of plasma free virus in healthy seropositive donors as well as neutralization of virus by anti CMV antibodies.[45]

Meyers et al reported that there is a high frequency of TT-CMV following transfusion of seropositive blood enriched for WBCs and demonstrated TT-CMV after granulocyte transfusion (57.1%, $p<0.001$) from seropositive donors transfused to seronegative recipients.[46]

Ping-Ing Lee et al suggested that fresh blood from seropositive donors was more infectious than stored blood (87% versus 17%, $p=0.01\%$).[47] However, not all studies have demonstrated an effect of product storage interval on the incidence of TT-CMV.

Blood Components implicated in TT-CMV

Blood Component	TT-CMV risk	Methods to prevent TT-CMV
Red cells	Yes	Screening, Filtration, Frozen-deglycerolized
Platelets	Yes	Screening, Filtration
Granulocytes	Yes	Screening
FFP	No	Not applicable
Cryoprecipitate	No	Not applicable
Clotting factors	No	Not applicable

The risk of TT-CMV also varies with different groups of transfusion recipients. Preiksaitis had suggested that the following factors may also predispose to TT-CMV: - sequential transfusion over a

long period of time as compared to large transfusion volumes given at one time, the use of HLA-matched donors, repetitive transfusions from the same donor and the degree of immunosuppression and cytokine expression profile in the host. This makes it very difficult to assign a specific risk per CMV seropositive unit transfused.[32, 33]

PATIENTS AT RISK FOR TT-CMV[32, 33]

Category A: *(Populations in whom the use of CMV-"safe" cellular blood products have been proven to reduce the incidence and morbidity of CMV infection using controlled trials)*

- Low birth weight infants born to seronegative mothers
- Seronegative recipients of seronegative donor bone marrow (allogeneic)
- Seronegative recipients of autologous bone marrow transplants

Category B: *(Populations at high risk of significant morbidity as the result of transfusion-acquired CMV infection, but the incidence of transfusion-acquired CMV infection in these populations has not been clearly documented or the benefit of using CMV-"safe" cellular blood products has not been proven)*

- Seronegative pregnant women requiring antepartum transfusion or intrauterine blood transfusions and seropositive women requiring intrauterine blood transfusions in the second trimester

- Low birth weight infants born to seronegative or seropositive mothers or other seronegative immunosuppressed patients requiring granulocyte transfusions

- Seronegative recipients of seronegative donor lungs and livers and possibly other organs

- Seronegative HIV-infected and AIDS patients and children born to HIV-infected mothers

Category C: *(Populations who may be at higher risk of transfusion-acquired CMV infection or its morbidity, but in whom the incidence or morbidity or transfusion acquired CMV infection is low or poorly documented)*

- Low birth weight infants born to seropositive mothers

- Infants with birth weights > 1,500 g born to seronegative mothers

- Neonates receiving ECMO (extracorporeal membrane oxygenation) and other neonates requiring extensive transfusion support (i.e. exchange transfusion, cardiovascular surgery)

- Seronegative recipients of seronegative donor kidneys and hearts

- Seronegative patients with malignant disease receiving chemotherapy

- Seronegative patients with hematological or genetic disorders requiring repetitive transfusions in whom bone marrow transplantation may be a future therapeutic option

- Seronegative patients experiencing major trauma or splenectomy

Category D: *(Populations in which the incidence and morbidity associated with transfusion acquired CMV infection is low, the use of CMV-"safe" cellular blood products is not indicated)*

- Infants with birth weights > 1,500 g born to seropositive mothers

- Other seronegative immunocompetent patients

The most well established patient groups at risk for TT-CMV include[48, 49]

1. Premature Low Birth Weight infants (<1250-1500g) born to seronegative mothers

2. Seronegative recipients of seronegative allogenic or autologous Bone Marrow Transplant (BMT)

3. Seronegative recipients of seronegative solid organ transplants

Immuno compromised patients have higher rates of CMV infection and CMV disease compared to immunocompetent individuals.[46]

Wilhelm et al showed that less than 1.2% of immunocompetent patients experienced transfusion transmitted CMV. But, the incidence of community acquired CMV infection is relatively greater (1.7% per patient year) than transfusion transmitted CMV in immunocompetent population.[50] Although transfusion transmitted CMV produces primary CMV infection in the immunocompetent transfusion recipient, it is of no more clinical significance than community acquired CMV infection. Thus at present there are no compelling reasons to provide non-immuno suppressed seronegative transfusion recipients with special components for the purposes of preventing transfusion transmitted CMV.

However, TT-CMV can be an important cause of morbidity and mortality in immuno compromised patients. Most studies suggest that 13-38% of these patients will contract CMV from transfusion of unscreened and unfiltered cellular blood components.[47, 51, 52]

The first manifestations are often a viral syndrome characterized by flu like illness, including fever, chills, malaise, leucopenia and thrombocytopenia. It can progress to CMV hepatitis, retinitis, interstitial pneumonitis, encephalitis and gastro enteritis. Progression to disease is more likely in patients with elevated viral loads.

Transplancental transmission of CMV to a developing fetus is an important viral cause of birth defects.[53] Fetal infection occurs in 40 to 50% of cases in which a seronegative mother contracts a primary CMV infection during pregnancy. CMV disease occurs in 5-15% of the infected infants, presenting with intra uterine growth retardation, deafness, mental retardation, blindness and thrombocytopenic bleeding.[54]

However, when mothers are seropositive before pregnancy, maternal antiviral immunity can limit congenital CMV infection and disease but they can also be at risk for lethal CMV infection, despite the transfer of humoral immunity especially in low birth weight infants.

Stagno et al stated that the rate of vertical transmission was approximately 1% among seropositive mothers.[54]

Ping-Ing Lee et al reported that seronegative children transfused with unscreened blood products had a 36% (14 of 39) incidence of TT-CMV.[47]

The morbidity in neonates weighing less than 1250-1500g with transfusion acquired CMV infection born to seronegative mothers ranged from 40% to 100% while the mortality ranged from 20% to 57% which is mainly due to their immature immune systems.[55]

Preiksaitis et al documented that primary CMV infection during pregnancy carries high risks of congenital fetal infection. As the primary maternal infection resulting from TT-CMV can in turn lead to fetal infection, it is prudent to provide CMV safe blood components to pregnant women who are seronegative.[56]

Marrow transplant recipients are at significant risk of morbidity and mortality from CMV infection. Up to one third of those patients who contract CMV infection can develop CMV pneumonitis, a frequently fatal complication.[46]

In seronegative recipients of seronegative marrow or autologous transplants, transfusion is the primary mechanism for CMV infection whereas in seropositive marrow recipients, CMV infection is usually due to viral reactivation.[52, 57]

The development of GVHD affects the incidence of CMV infection and disease in BMT patients. Miller et al reported that CMV infection occurred in 42% of allogenic bone marrow transplant patients who developed GVHD and in only 20% of those who did not develop GVHD.[57] The incidence of CMV interstitial pneumonitis in seronegative recipients of allogenic BMT was significantly greater than the autologous BMT particularly if the allogenic recipients developed GVHD.

Solid organ transplant recipients are also susceptible to CMV infection and disease. In contrast to Bone marrow transplantation, the most important source of CMV infection is the donor organ, with TT-CMV being less significant.[58, 59]

In seronegative recipients of seronegative organs, transfusion of unscreened blood products has been associated with an incidence of CMV ranging from 15% to 20%.[55]

Among organ transplant recipients, those receiving heart, heart-lung, liver and pancreas transplants usually require numerous transfusions and thus have an increased risk (up to 33%) of TT CMV. Hillyer et al showed that even in heavily transfused organ transplant recipients, the use of seronegative blood products could effectively prevent transfusion transmitted CMV.[60]

Previously, CMV was not considered to be oncogenic but recent studies show that they too play a role in carcinogenesis. Bongers et al reported that CMV promotes development of intestinal dysplasia and cancer in transgenic mice and suggest that CMV infection may facilitate development of intestinal neoplasia in humans. Centre for molecular medicine, Sweden revealed the frequent presence of CMV genome in certain malignant tumours. Whether CMV is causative or simply represents an epiphenomenon of malignant tumors requires further elucidation.[61] If this has been proved, considerable stress will be placed on blood banks to provide CMV free components.

LABORATORY DIAGNOSIS

Accurate detection of CMV infection enables the identification of transfusion recipients at risk for CMV infection, as well as blood donors whose components are potentially infectious.

CMV may be routinely detected in infected individuals either by the direct identification of infectious virus or viral nucleic acid proteins or indirectly by the serological measurement of the CMV specific antibody response.

The standard approach for identifying a previously infected individual is through detection of anti CMV antibodies. Serologic assays have been developed in multiple configurations, including indirect hemagglutination, complement fixation, solid phase fluorescence immuno assay, enzyme immuno assay (EIA), latex or particle agglutination and solid phase red cell adherence, although the first three of these techniques are no longer frequently used.[20]

Current transfusion practice is to use EIA designed to detect CMV specific antibody. These techniques are used qualitatively and have several distinct advantages for transfusion services as they are sensitive (93%), specific (95%), rapid and can be automated for high sample throughput.[60]

EIA systems that detect both CMV specific IgG & IgM are to be preferred as the IgM component may improve identification of those donors with acute, but sub-clinical CMV infection.

The incidence of new CMV infection is estimated to be 1% per annum in adults. However, anti-CMV antibodies may not be detected by serology until 6 to 8 weeks after primary infection and serology cannot accurately identify or quantitate the extent of active CMV infection (Fig 2).

Poor antibody responders to CMV may also be unreactive leading to additional false negative EIA test results. Therefore apparently CMV seronegative blood components may contain and transmit infectious virus. This accounts for occasional TT CMV infections seen despite the use of seronegative products.

An alternative to serological testing is direct detection of the virus or its component nucleic acid / proteins. Viral culture is relatively insensitive but unequivocally determines whether or not infectious virus is present and therefore provides an excellent inferred measure of blood product infectivity. However, prolonged assay times (>48 hours) make this approach impractical for the routine screening of blood donations.

Antigenemia assays, frequently based on detection of the CMV tegument phosphoprotein *pp65*, are used to monitor clinical infection in patients. This method is used for early quantitative detection of CMV infections, allowing the institution of preemptive (presymtomatic)

antiviral therapies. Owing to their relative insensitivity at low viral loads, they are of limited value in determining the CMV status of latently infected blood donors.

The recent introduction of quantitative PCR assays for CMV may provide a more rapid, sensitive and specific predictor of patient at risk for CMV disease.[62] Advantages of the PCR method included reduced turn around time, smaller sample requirements, simplified specimen processing, improved stability of specimens. PCR can be useful in detecting CMV DNA either from the plasma or WBC during window period (Fig 2).

PREVENTION OF TT-CMV

The incidence of TT-CMV as well as that of other untoward effects of transfusion can be reduced by limiting transfusion to appropriate, clinically indicated circumstance. However, when transfusions are necessary, the most common approaches to decrease the risk of TT-CMV are the use of blood components that are considered CMV safe.

I) SERONEGATIVE BLOOD COMPONENTS

The exclusive use of seronegative units in immunocompromised adult seronegative recipients of allogenic seronegative bone marrow transplants decreased the incidence of CMV and severity of resulting CMV disease as compared to the use of unscreened blood products.

However, the use of seronegative units was only beneficial in seronegative recipients of seronegative donor marrow. Seronegative recipients of seropositive marrow transplants had a 46% incidence of CMV infection when transfused with unscreened blood which was not significantly different from the 32% incidence when these patients received seronegative blood products.[52]

The potential activity of screening donors for CMV antibody to prevent TT-CMV infections and disease was highlighted by a series of important studies over the past three decades.

Luthardt & Colleagues in 1971 showed that 0 of 20 seronegative exchange transfused infants receiving seronegative donor blood acquired CMV infection, whereas 8 of 15 (53%) seronegative infants who were exchange transfused with CMV seropositive blood became infected.[63]

Yeager & associates published that 0 to 90 seronegative infants receiving seronegative blood became infected with CMV; in contrast, 10 of 74 (13.5%) seronegative infants receiving seropositive blood were infected, 5 of whom manifested serious or fatal CMV infections.[51]

Despite exclusive use of seronegative units for transfusion, up to 4% of susceptible recipients have acquired CMV.[64] Several explanations for the failure of antibody screening to eliminate TT-CMV infections are possible, including the presence of strain variants undetected by current antibody assays, insensitive antibody assays, waning antibody in formerly seropositive donors (Seroreversion) and transmission during the antibody negative window phase of infection. Alternatively some of these infections may not be transfusion related, but instead result from non parental spread that occurs in the general population.

Alternatively, some donors may have been in the 6 to 8 week window phase following primary CMV infection during which anti-CMV antibodies cannot be detected reliably.[31] During this period there are high peripheral blood viral loads, suggesting that transfusion from these seronegative donors may be infectious.

Although most CMV antibody tests are based on the broadly reactive strain AD169, it is theoretically possible that currently used tests might not detect some infections caused by variant strains. There is, however, no solid evidence to substantiate this contention. Antigenic variation among CMV strains was documented by cross-neutralization studies shortly after the virus was first isolated, but the amount of variation was not sufficient to warrant designating different serotypes. Thus strain variation is unlikely to account for the occurrence of break through infections in high risk patients given seronegative blood components. CMV is thought to establish a latent infection with periodic reactivations, providing an immune stimulus that ensures life long antibody positivity in the vast majority of infected persons.[62]

II) FILTERED BLOOD COMPONENTS

The difficulties in maintaining a sufficient inventory of CMV seronegative blood components, due to its high prevalence, motivated efforts to identify alternative strategies to provide CMV safe components for susceptible patients. Removal of WBCs from components was an attractive approach to mitigate TT-CMV. Current generation of leukofilters have excellent leukocyte removal efficiency (99.99% -3 to 4 $\log_{10}$ reduction) as compared to the previous generation filters (90-96%).[65]

The primary mechanism of leukocyte removal is the charge-based adhesion of negatively charged leukocytes to the filter material by Vander Waals and electrostatic forces. This adhesion is an active process and has the advantage of larger pore size, by which a subsequent higher flow rate is possible in the filter. The surface charge of the filters can be modified by coating the filter material with methacrylate polymers, to create a stronger positive charge and hence increase the efficiency of the filter.[66]

Alternatively, platelets and red cells can be prepared from donors by apheresis procedure, resulting in components with 10^5 to 10^6 residual leukocytes. This level of leukoreduction has been shown to significantly reduce the incidence of TT-CMV.[67, 68]

Prestorage leukoreduction may reduce the risk of CMV transmission by blood products not only by reducing the number of latently infected cells infused but also by reducing the probability of initiating CMV reactivation events driven by cytokine release from donor leukocytes in a blood component before infusion.

The American Association of Blood Banks (AABB) has suggested that residual leukocyte levels less than 5×10^6 make a blood product CMV-"safe". In its theoretical limit, the infusion of a single

latently infected donor leukocyte may be sufficient to infect a susceptible recipient. However, in practical terms, because the pathogenesis of transfusion-acquired CMV infection involves a complex interaction between donor and recipient factors, leukoreduction to less than 5×10^6 leukocytes in a blood product may make the sequence of events required to reactivate CMV from latency in almost all transfusion recipients so improbable, that the blood product could be considered CMV-"safe."

Although the AABB guidelines state that seronegative and leukoreduced units are equivalent for prevention of TT-CMV, other panels disagree. A Canadian consensus reached a conclusion, when the majority of the panel agreed that, seronegative blood components should continue to be provided to at risk patients despite implementation of universal leukoreduction in Canada.[69]

Wu Y et al reported that transfusion transmitted CMV may still occur in the era of universal leukoreduction.[70]

Lipson et al stated that Plasma viremia, if present, would not be diminished by leukoreduction and could explain CMV transmission events following use of leukoreduced components.[71]

In order to directly compare the incidence of TT-CMV with seronegative and filtered components, Bowden & Colleagues prospectively randomized seronegative bone marrow transplant recipients to receive transfusions of either seronegative blood units or unscreened filtered blood products. The incidence of CMV infection was comparable in both groups (1.3% in the seronegative arm Vs 2.4% in the leukoreduction arm). However, the incidence of CMV disease and CMV related mortality was slightly higher in the leukoreduced arm of the study (0% Vs 2.4%) p=0.03.[64]

Nicholas et al reported the longest clinical study that assessed the efficacy of serological screening versus WBC reduction. This investigator concluded that CMV seronegative components is superior to WBC reduced components in preventing TT-CMV infection and the abandonment of CMV seronegative inventories in an era of universal WBC reduction is probably immature.[72]

The University Health system Consortium (UHC) consensus recommends that leukoreduced components may be considered equivalent to seronegative components but they may not be applicable to all immunosuppressed individuals. A decision to order leukoreduced components should be based on patient's immune status, underlying co-morbidities and overall physical condition.[73]

Smith et al reported that though both seronegative and leukoreduced products were considered equivalent by many institutions, the reported practices for specific patient populations did not match this view of equivalence, with many patient populations preferentially receiving CMV seronegative components. Fetal and neonatal populations were more likely than other patient populations to receive CMV seronegative products to reduce the risk of TT-CMV.[74]

Canadian consensus concluded that CMV serologic testing might add to the benefit of leukoreduction in those patients at highest risk of developing CMV disease and there is no evidence that abandoning serologic testing does not lead to a slight, but clinically important increase in CMV transmission.[72]

The Council of Europe has endorsed the use of leucodepleted cellular blood components as a safe substitute for CMV seronegative blood components where the latter are unavailable.[20]

Malte et al reported that donors who were seropositive for atleast 1 year did not show the presence of CMV DNA. Hence, blood products from these donors can be leukoreduced and transfused for immunocompromised patients when seronegative blood is not available.[75]

When assessing the overall cost-benefit of leucodepletion Vs serological screening, the additional benefits of leucodepletion should also be taken into account. Leucodepletion, as an alternative to CMV testing, has been shown to prevent primary allosensitization in > 97% of transfusion recipients and FNHTR. By contrast, the sole benefit of CMV antibody testing is prevention of CMV transmission. Disadvantages of serological testing include selective screening for certain patients, associated clerical errors and insufficient inventory of CMV seronegative products, particularly in emergencies.[20]

III) NUCLEIC ACID AMPLIFICATION TESTING (NAT) SCREENED COMPONENTS

NAT is currently used to screen for HIV and HCV in blood donations suggesting that NAT for CMV DNA may be a useful adjunct to serology and filtration in preventing TT-CMV.

However, CMV NAT poses problems not encountered with HIV AND HCV screening. Because most seropositive donors who are immunocompetent are remotely infected (>6 months), they are likely to have peripheral blood viral loads near or below the limits of detection of even sensitive NAT assays. Assays sufficiently sensitive to detect these low viral loads may be subject to problems including non-specific

amplification of background DNA. This high DNA content may adversely affect the signal to noise ratio of NAT assays. Thus it is not surprising that previous applications of NAT to detect CMV DNA in healthy blood donors have produced conflicting results.[18]

Marc Mendelson et al have identified CMV DNA in both seropositive and seronegative donors.[76] Andreas et al identified CMV DNA in 5 of 27 of healthy blood donors.[77] In contrast, Bitsch et al and Smith et al have been unable to identify CMV by PCR in peripheral blood from seropositive or seronegative donors.[78, 79] They concluded that CMV genome copy number in healthy individuals is beyond the detection limit of PCR technology. David Hudnell et al reported CMV DNA in only 1% of the healthy donors.[80]

To address the inconsistent results of earlier studies, a multicentre trial was performed by Roback JD et al to directly compare the performance characteristics of CMV PCR assays when applied to healthy blood donors. They found only 0.5% of samples from healthy CMV seropositive blood donors to have reproducibly detectable CMV DNA loads when using extremely sensitive PCR assays. Based on these results, the current CMV PCR assays do not appear to improve upon the ability of serological screening to prevent TT-CMV.[81]

Dumont & Coworkers have published a provocative study suggesting that CMV DNA in blood may vary seasonally with higher levels of viremia coinciding with increased tree pollen concentration. If the hypothesis is confirmed, it is possible that higher rates of plasma CMV DNA might be detected during periods when immunologic stimulation of donors is enhanced because of allergen exposure.[82]

Boom et al observed that the CMV DNA present in the plasma and serum of renal transplant recipients with primary CMV infection was highly fragmented and therefore unlikely to represent incubating infectious virus.[83] If this finding can be validated by others, it would allay concerns expressed by Drew et al that plasma viremia may be source of residual CMV transmission when WBC reduced or seronegative cellular blood components are used.[38] It would also explain the clinical observation that plasma and plasma products do not appear to transmit CMV.

It should be noted that minimal viral load required for TT-CMV has not been determined and it must be assumed at present that any CMV-seropositive or CMV DNA positive unit is potentially infectious.

With standard serologic assays, both uninfected donors and those in the window phase of a CMV infection test as seronegative. Some

window phase donors have high plasma & WBC-associated viral loads and thus although seronegative, their blood may be infectious on transfusion.[84] NAT may serve as a useful adjunct to serology and filtration in those who are in window period.

IV) FROZEN DEGLYCEROLISED COMPONENTS

The exclusive use of frozen-deglycerolized red cells markedly reduces the incidence of TT-CMV. Robert et al stated that only 0.8% of the seronegative bone marrow patients got seroconverted after receiving frozen deglycerolized RBCs. However, given the labor intensive nature of preparing frozen–deglycerolized red cells and its cost, this approach is rarely used.[85]

V) WASHED COMPONENTS

It is unclear whether washing of components can adequately decrease the incidence of TT-CMV. Grundy et al[86] reported that transfusion of washed cells resulted in an 11% incidence of CMV in seronegative neonates while Gail Demmeler et al[87] reported 1.3% incidence. The variable rates may be explained by the variations in washing protocols. Still, most authorities do not equate washed RBCs with seronegative components for the prevention of TT-CMV.

VI) IMMUNOGLOBULIN PREPARATION AND ANTIVIRAL DRUGS

A meta-analysis of prophylactic IVIg in BMT patients confirmed that passive CMV antibodies could significantly reduce fatal CMV infection, CMV pneumonitis and total CMV mortality. However, the efficacy of IVIg in preventing TT-CMV is more difficult to assess. There was no significant effect of IVIg on CMV infection and disease, when seronegative patients were transfused with seronegative blood. Further, IVIg was ineffective in preventing TT-CMV in seronegative patients who received seropositive granulocyte transfusion.

Ganciclovir can provide effective prophylaxis against CMV disease. However, it can cause marrow suppression predisposing to opportunistic infections. Broers et al reported severe neutropenia in 33% of treated patients.[88] These patients experienced increased mortality due to other infections despite control of CMV infection.

When compared to the proven efficacy of seronegative or filtered units in preventing TT-CMV, the use of IVIg and antiviral agents would be more expensive and possibly not as effective, and thus these interventions cannot be recommended for abrogation of TT-CMV.

VII) GAMMA IRRADIATION AND PATHOGEN INACTIVATION

Gamma-Irradiation of cellular blood components to minimum doses of 2500 cGy (25 Gy) to the mid-plane of the container and 1500 cGy to all other parts is used to prevent TA-GVHD. This prevents thymidine incorporation by lymphocytes after mitogenic stimuli. A 500-cGy dose may suffice to prevent the physiologically relevant proliferation in mixed lymphocyte culture. Doses <5000 cGy do not affect RBC, platelet or granulocyte function and survival adversely. But to prevent CMV transmission, Gamma-Irradiation cannot be used because the dose needed to inactivate the virus can damage blood cells.[89]

As opposed to current approaches such as the use of seronegative and filtered components, the application of pathogen inactivation technology to cellular blood components carries the potential for completely preventing TT-CMV as well as eliminating the transmission of other infectious agents. In the presence of ultra violet light, psoralen based compounds such as 8- methoxypsoralen can inactivate a spectrum of pathogens, including viruses and bacteria in blood components. It has effectively prevented transfusion transmitted CMV in a murine transfusion model and clinical trials are warranted in humans.[90]

MATERIALS AND METHODS

STUDY METHOD

This prospective study was conducted over one year period from 2009-2010 in the Department of Transfusion Medicine, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai. A total of 180 voluntary blood donors were selected. The study was approved by the ethical committee of the Tamil Nadu Dr. MGR Medical University, Chennai. The donors were classified as higher, middle and lower socioeconomic status based on Kuppusamy classification.

SAMPLE COLLECTION

Five ml of blood from each donor was collected from the collection bag into a sterile capped tube. It was then centrifuged and plasma was separated and stored as 2 aliquots at -80°C till further use.

INCLUSION CRITERIA

- Donors who are eligible for blood donation as per the NACO guidelines.
- Donors reactive for any of the existing mandatory test, as per the NACO guidelines, are also included.
- Donors who are willing to participate in the study by giving written consent.

EXCLUSION CRITERIA

All voluntary donors not willing to give consent to participate in the study

STATISTICAL ANALYSIS

Statistical analysis was done with “Chi-square Test” using statistical software packages (Microsoft Excel, SPSS15). Groups were assumed to differ significantly when the probability (p value) was less than 0.001 (1% Level) or 0.05 (5% level).

METHOD OF SCREENING

The samples that were frozen earlier were thawed and used. Sera were tested for IgG and IgM CMV by the enzyme-linked immunosorbent assay (ELISA) test. The CMV-specific antibodies were studied by the commercial Diagnostika Nord CMV IgG ELISA Kit (Fig 3) and CALBIOTECH CMV IgM ELISA Kit (Fig 4). This is based upon the use of micro titration wells coated with purified antigen. All steps were done according to the manufacturer's instructions. Reading was taken at 450nm wavelength using a microplate reader (Fig 5, 6).

CMV DNA DETECTION

The detection of CMV DNA in the CMV seronegative samples was done by real time PCR using Roche light cycler (Fig 7). The DNA was extracted from the plasma by Shanghai ZJ Bio-Tech CMV Real time PCR Kit.

THE PROTOCOL FOR THE DNA PURIFICATION FROM PLASMA

Procedure

DNA extraction buffer supplied in the kit is thawed thoroughly and spin down briefly in the centrifuge before use.

1) Pipette 50µl serum or plasma to a 0.5ml tube, add 50µl DNA extraction buffer, and close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

2) Incubate the tube for 10 minutes at 100°C.

3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for the template of the PCR.

PROCEDURE OF DNA AMPLIFICATION

PCR Primers

Forward Primer : 5' GAG GAC AAC GAA ATC CTG TTG GGC A 3’

Reverse Primer : 5' GTC GAC GGT GGA GAT ACT GCT GAG G 3’

Probe : 5’ GGA CTA CCT CTT CAA ACG CAT GAT TGA C 3’

(3’fluorescein label)

Real time cycles condition

Reaction mixture was prepared as follows:

Fast start DNA master hybridization probe	-	2µl
Forward primer	-	2µl
Reverse primer	-	2µl
Probe	-	2µl
Magnesium chloride	-	3.2µl
Water	-	3.8µl

15µl of the reaction mix is dispensed straight into the Light cycler capillary. 5µl of the DNA extract, positive/ negative control is added. Capillaries are capped and spinned at 1000rpm for 10seconds to deposit the reaction mix at the base of the reaction capillary. It is transferred to the Roche Light cycler.

DNA is amplified as follows:

Denaturation done at 95^{o} C for 30 sec. Amplification at 60^{o} C for 45 seconds and extension at 72^{o} C for 30 seconds

Finally melting curve analysis was done. This is a step built into the software of real time cycles.

RESULTS

TABLE 1

Gender Distribution of the Study Group

Sex	Number of donors	Percent %
Male	137	76.1
Female	43	23.9
Total	180	100.0

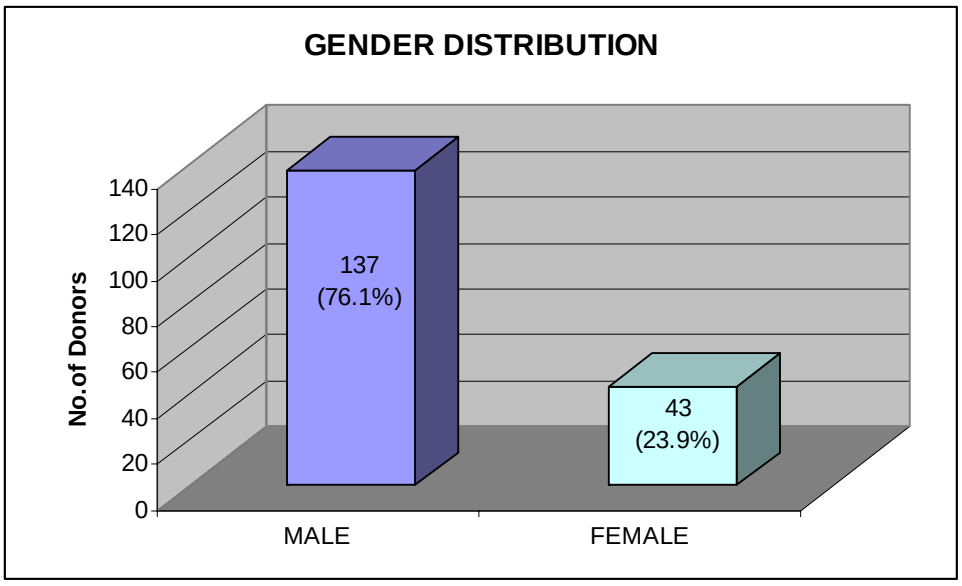


Fig 6

TABLE 2

Age Distribution of the Study Group

Age group In years	Number of Donors	Percent %
18-20	60	33.3
21-25	62	34.4
26-30	34	18.9
31-35	14	7.8
36-40	8	4.4
> 40	2	1.1
TOTAL	180	100.0

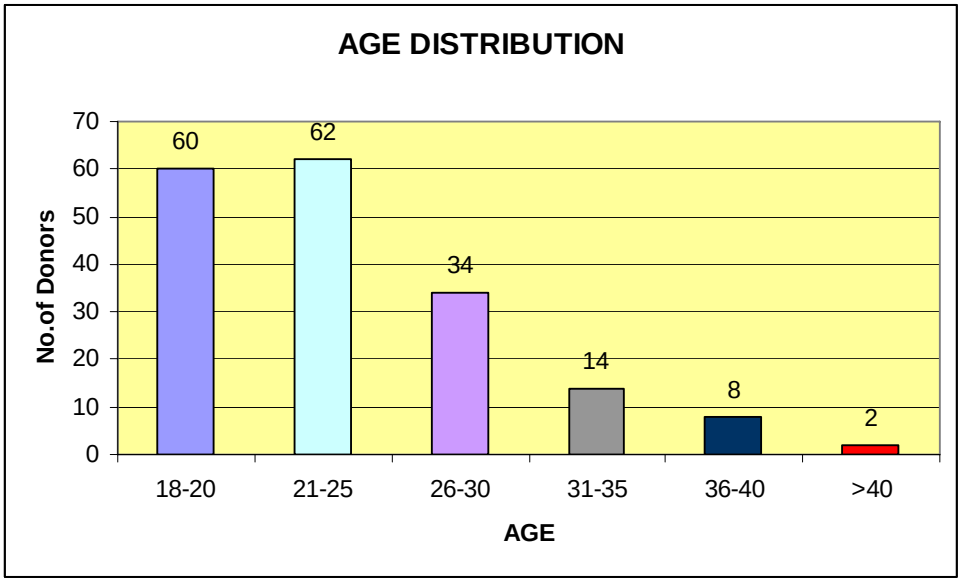


Fig 7

TABLE 3

Distributions On The Basis Of Occupation

Occupation	Number Of Donors	Percentage%
Professionals	65	36.1
Students	74	41.1
Clerical job	24	13.3
Coolie	17	9.4
Total	180	100.0

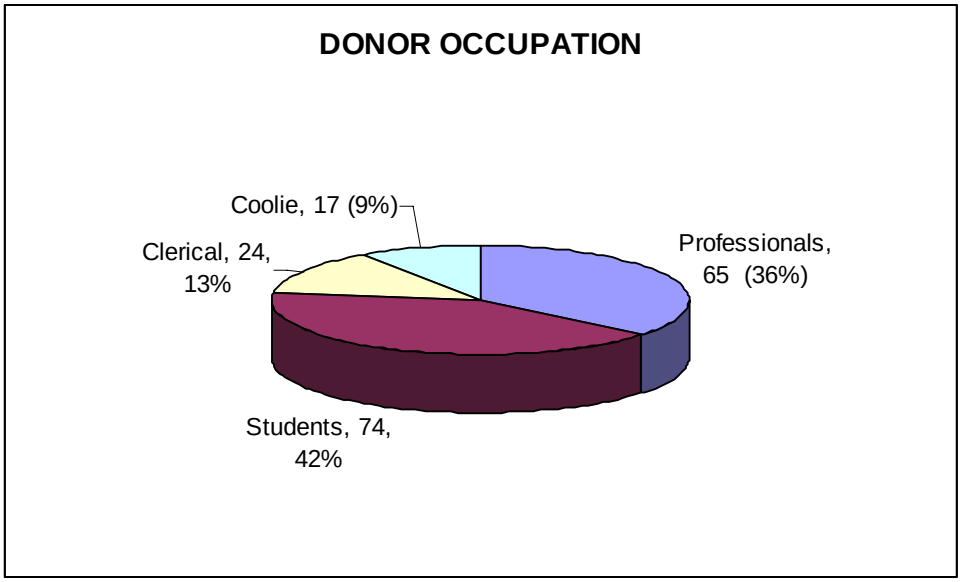


Fig 8

TABLE 4

Distribution of Blood Group

Blood Group	Number of Donors	Percent %
A Positive	39	21.66
A Negative	4	2.2
B Positive	55	30.55
B Negative	3	1.7
O Positive	64	35.6
O Negative	4	2.2
AB Positive	11	6.1
AB negative	0	0.0
Total	180	100.0

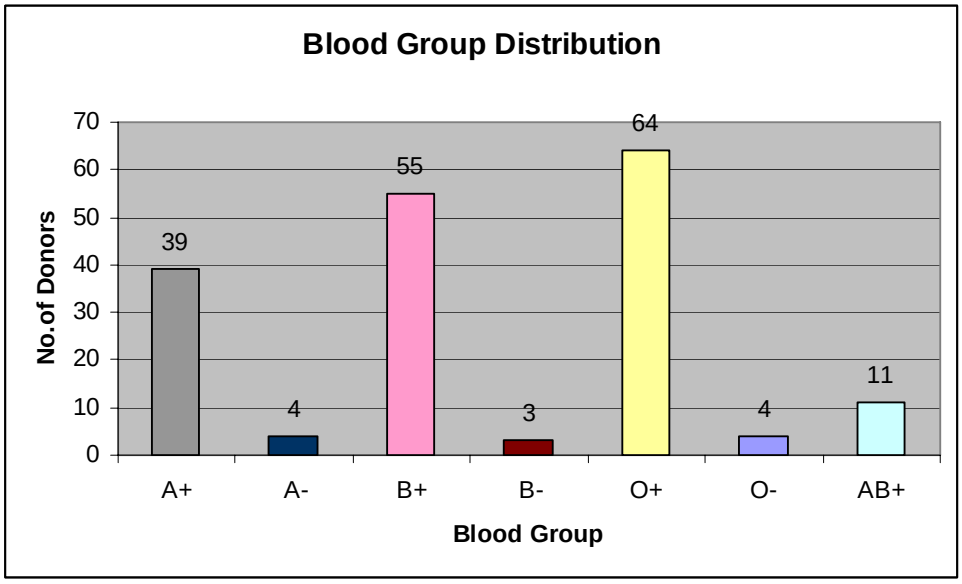


Fig 9

TABLE 5

Distribution On The Basis Of Socioeconomic Status

Socioeconomic status	Number of donors	Percentage %
High	23	12.78
Middle	131	72.77
Low	26	14.44
Total	180	100.0

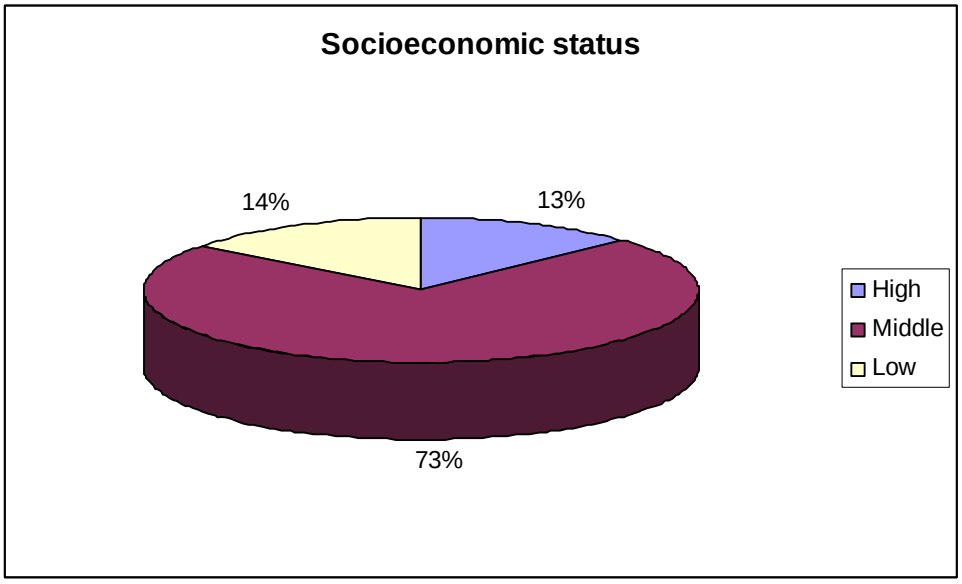


Fig 10

TABLE 6

Transfusion Transmitted Infection Status

TTI Status	Number of donors	Percentage %
Reactive	1(HbsAg)	0.56
Non-Reactive	179	99.44

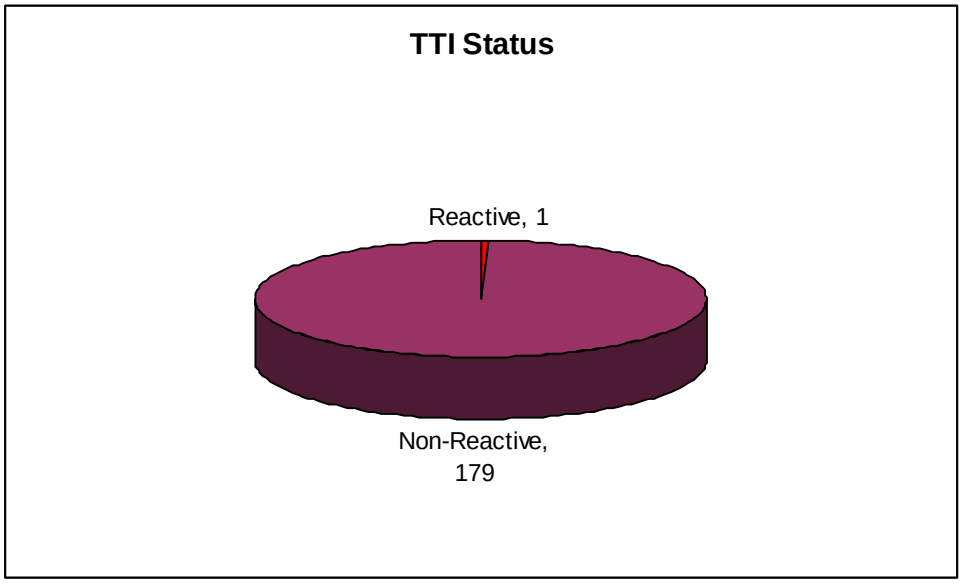


Fig 11

TABLE 7

Anti-CMV Antibody screening by ELISA

Anti-CMV antibody	Positive	Negative
IgG	167	13
IgM	0	180

P=0.000

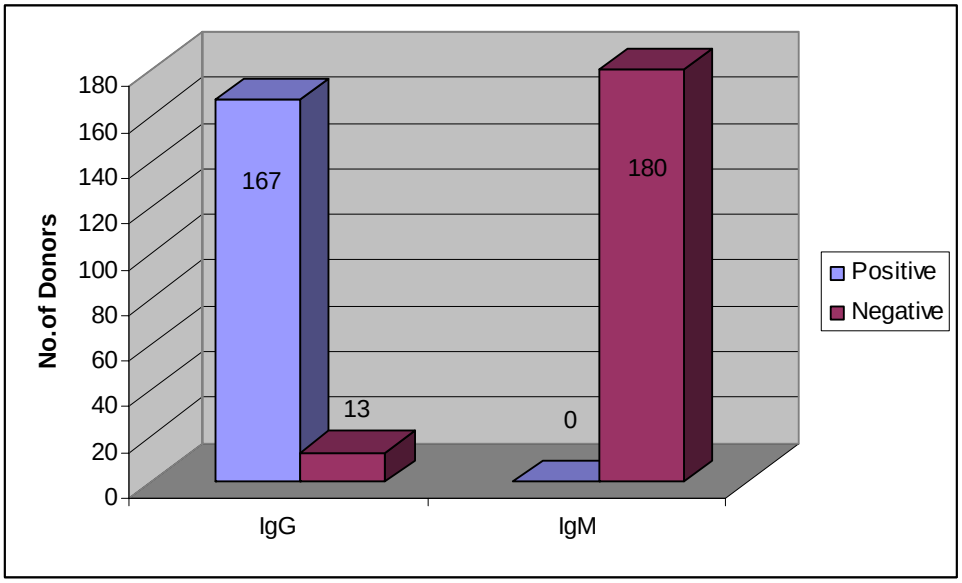


Fig 12

TABLE 8

PCR results in IgG seronegative donors

IgG Anti-CMV Negative	13
PCR positive	0

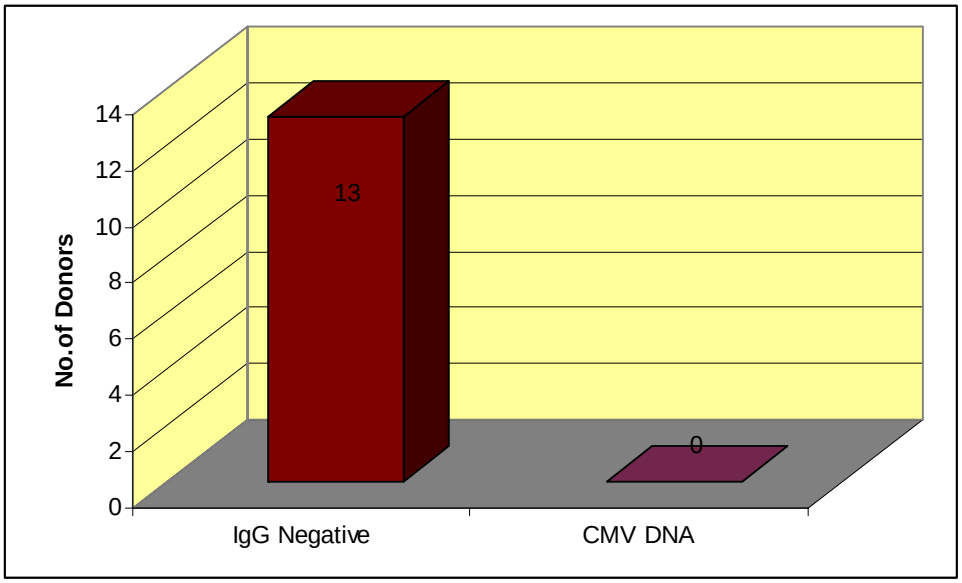


Fig 13

TABLE 9

Age Distribution of IgG seropositives

Age group In years	IgG seropositive donors (Total donors)	Percent %
18-20	56(60)	93.33%
21-25	58(62)	93.54%
26-30	30(34)	88.23%
31-35	13(14)	92.85%
36-40	8(8)	100%
> 40	2(2)	100%
TOTAL	167(180)	92.8%

P>0.05

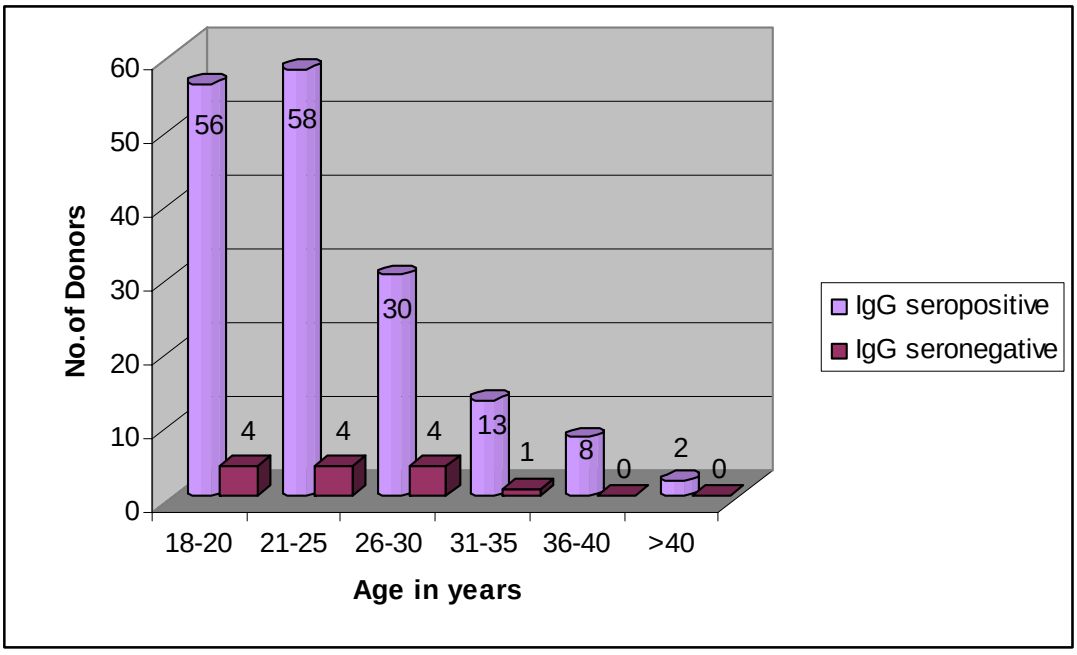


Fig 14

TABLE 10

Gender Distribution of IgG Seropositives

Sex	IgG seropositive donors (Total donors)	Percent %
Male	124(137)	90.51%
Female	43(43)	100%
Total	167(180)	92.8%

P=0.036

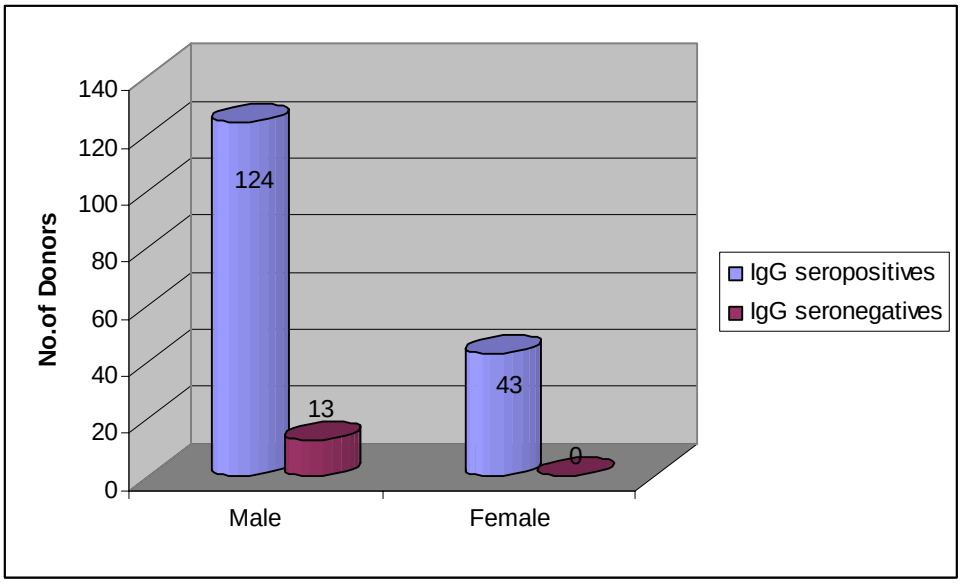


Fig 15

TABLE 11

IgG seropositives on the basis of socioeconomic status

Socioeconomic status	IgG seropositive donors (Total donors)	Percentage %
High	19(23)	82.6%
Middle	122(131)	93.12%
Low	26(26)	100%
Total	167(180)	92.8%

P=0.041

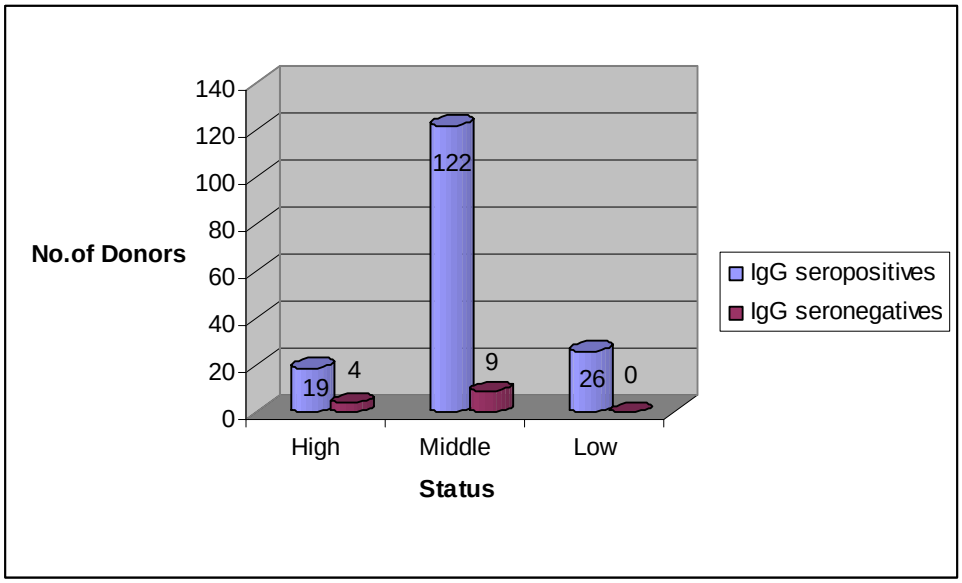


Fig 16

Demographic analysis showed, of the 180 donors, 137 (76.1%) were males and 43 (23.9%) were females. (Table 1; Fig 6)

Age distribution among the blood donors were 33.3% in 18-20 years, 34.4% in 21-25 years, 18.9% in 26-30 years, 7.8% in 31-35 years, 4.4% in 36-40 years, 1.1% in >40 years. (Table 2; Fig 7)

Percentage distribution of blood donors on the basis of occupation were 36% of Professionals, 42% of students, 13% of clericals, 9% of coolie. (Table 3; Fig 8)

Blood group distributions among the donors were 21.66% of 'A' positive, 30.55% of 'B' Positive, 35.6% of 'O' Positive, 6.1% of 'AB' positive. Rh D negative donors constitute about 6.1%. (Table 4; Fig 9)

Most of our donors belong to middle socioeconomic status (78.33%) followed by high (12.78%) and low (8.89%). (Table 5; Fig 10)

Among 180 voluntary blood donors, only one was found to be reactive for Hepatitis B Surface Antigen (HbsAg). (Table 6; Fig 11)

CMV IgG antibody screening by ELISA showed that 13 were negative and 167 were positive, giving an overall CMV prevalence rate of 92.8%. None of the 180 blood donors were reactive for CMV IgM antibodies by ELISA test. (Table 7; Fig 12)

Of the 13 IgG seronegative blood samples, none were found to contain CMV DNA by real time PCR (RT-PCR). (Table 8; Fig 13)

DISCUSSION

The present study was undertaken to define the seroprevalence of CMV infection among voluntary blood donor population, since voluntary donors are expected to provide the major source of most blood transfusion requirements. Our blood centre has 100% voluntary blood donation, hence the present study comprised only of voluntary blood donors.

Kaur et al reported that voluntary donations need to be encouraged as voluntary donors are safer than replacement donors.[91]

As is evident from the results shown in our study, about 167 out of 180 (92.8%) donors were positive for IgG anti-CMV antibody, suggestive of past exposure to infection. (P=0.000; 95%CI 1.0340-1.1104)

IgG Seroprevalence of cytomegalovirus in various studies:

STUDY	PLACE	IgG SEROPOSITIVITY
Atul Kothari et al[92]	New Delhi	95% (n=200)
Chaudhari et al[93]	Pune	87.9% (n=431)
Mukundan et al[94]	Vellore	92% (n=212)
Adjei et al[95]	Ghana	93% (n=264)
Akinbami et al[96]	South Africa	96.7% (n=122)

STUDY	PLACE	IgG SEROPOSITIVITY
Pultoo et al[8]	Mauritius	93.5% (n=584)
Ahmed et al[97]	Malaysia	97.6% (n=172)
Uslu et al[98]	Pakistan	85.4% (n=89)
Amarapal et al[99]	Thailand	70.75% (n=441)
Robert et al[100]	Missouri, USA	59% (n=223)
Per Ljungman[101]	Netherlands	51.8% (n=23048)
Seale et al[102]	Australia	57% (n=3593)

From the above table, it is evident that our study results are in concordance with the results of developing countries. In contrast, the IgG seroprevalence is comparatively lower in developed countries.

On the other hand, none of the donors were positive for IgM anti-CMV antibody, indicating the absence of primary infection. Our IgM anti CMV seropositivity was similar to the study done by Kothari et al[92] in New Delhi, Pal SR et al[103] in Chandigarh, Adjei et al[95] in Ghana.

In contrast, Akinbami et al reported 19.5% of the donors (n=122) to be positive for IgM anti-CMV antibody in South Africa.[96] Amarapal et al[99] reported 9.52% of Thai blood donors to be positive for IgM anti-CMV antibody while Moniri et al[10] reported 2.3% IgM seropositivity in Iran. These reflect donors with recent infection or reactivation.

In our study, about 93.33% of the donors (56 out of 60) aged between 18-20 years were seropositive for CMV, as against 93.54% (58 out of 62) in 21 to 25 years, 88.23% (30 out of 34) in 26 to 30 years, and 92.85% (13 out of 14) in 31-35 years, and 100% in 36-40 years (8 out of 8) and >40 years (2 out of 2). There was no statistically significant difference (P>0.05) in the CMV IgG status in different age groups. This differs with study done Smith et al who reported that the prevalence of the antibody to CMV increases with age. They reported that the prevalence of the antibody increased from 81% (in 21-30yrs) to 88% (in 41-50yrs).[77] Though there was 100% seropositivity above 36 years in our study, it may be likely due to smaller number of blood donors in that age group. So this may not be a significant finding with the relatively low sample size in that age group.

The IgG seropositivity among male donors in our study was 90.51% (124 out of 137) while it is 100% (43 out of 43) in females. There was a significant statistical difference (P=0.036) in seroprevalence between sexes. This is similar to the study done by Pultoo et al who reported that the seropositivity was 93.1% in males and 100% in females.[8] Per Ljungman et al found that the risk of seropositivity increased with females (p<0.001).[101]

About 82.6% (19 out of 23) of the donors in higher socio economic group are found to be seropositive for CMV while 93.12% (122 out of 131) in middle and 100% (26 out of 26) in lower socio economic group are found to be seropositive. There was a statistically significant difference (P=0.041) in IgG status in different socioeconomic status. This is in concordance with the study done by Pia et al in Finland who reported that the seropositivity increases in lower socio economic group when compared to higher socioeconomic group (from 60.9% to 76.4%; p=0.004).[104] Sheevani et al in Punjab also reported that there was a decline in seropositivity with rising socioeconomic status.[105] De Jong et al reported that infection with CMV is endemic in the developing countries and in areas of low socioeconomic conditions, which is predominantly related to the closeness of contacts within these populations.[106]

Since all the donors included in our study were voluntary blood donors, the prevalence of infections (HIV, HBV, HCV, Syphilis and Malaria) that are screened for mandatory tests in the study group were low. Only one among 180 blood donors was found to be positive for HbsAg. This donor was also positive for IgG anti-CMV antibody. Bayram et al reported that CMV is more common in chronic HBV and HCV patients. CMV infection was demonstrated in 52.3% of chronic HBV, and 36% of chronic HCV patients.[107]

No correlation was observed between IgG seropositivity of CMV and either educational level, marital status or the blood groups. This is similar to the findings of Moniri et al[10] and Kothari et al.[92]

To address the issue of window period, the 13 seronegative samples were subjected to RT-PCR for detecting CMV DNA. But none was found to contain CMV DNA. This is similar to the study done by Bitsch et al on 116 CMV seronegative donors, which showed absence of amplifiable DNA in all.[78] Greenlee et al showed that CMV DNA was undetectable by real time PCR in both seronegative (n=93) and seropositive donors (n=110).[108]

However, our results differ from those of Larsson et al who found amplifiable CMV DNA in seronegative donors (19 of 140).[109] But, the same authors in another study could not able to detect CMV DNA in a different study group of 20 seronegative donors.[81] Nitsche et al found 5 of 22 seronegative donors to have CMV DNA.[110] The discrepancies might be explained by the use of different methods of extraction and DNA amplification. Roback JD et al had done the first multicentre trial to compare the sensitivity of PCR techniques and showed that some of the positive results in seronegative donors were due to spurious amplification of background genomic DNA in the samples. They also

reported that at low viral concentrations in seropositive donors, not all aliquots of a given sample would contain sufficient target to be detectable by PCR, which may explain indiscriminate results in various studies.[81]

Ziemann et al reported that CMV DNA was detectable in peripheral blood for up to 269 days after the primary infection. Clearance of CMV DNA from blood correlated with clearance of IgM antibodies and the development of IgG antibodies.[111]

Due to high seropositivity (92.8%) in our study, discarding blood positive for IgG anti-CMV antibody is not feasible. The council of Europe has endorsed that alternatives like leukoreduced blood products can be used when seronegative blood is not available. However, CMV seronegative components should continue to be used in preference to leukoreduced components for the transfusion needs of patients who are at increased risk of CMV disease.[20]

Future projects

- To study the frequency of TT-CMV infection in seronegative patients when seropositive and seronegative blood components were transfused
- To study the effect of leukofilters in TT-CMV transmission

SUMMARY AND CONCLUSION

- The seroprevalence of IgG anti-CMV antibody among voluntary blood donors in Chennai is 92.8% (167 out of 180). The high prevalence indicates the endemicity of infection, and this perhaps is related to socio-economic and environmental factor.

- None of the IgG CMV seronegative donors were found to contain CMV DNA by RT-PCR. However, in order to rule out CMV infected donors in window period, it is imperative to confirm all cases of seronegative donors by RT-PCR.

- IgG seropositivity is found to be significantly high in lower socioeconomic status and female population.

- Age, educational qualification, marital status and blood group do not have any correlation with IgG CMV seropositivity.

- None of the donors were found to be IgM anti-CMV antibody positive. Considering the cost being high and IgM antibody positive donors seldom found, screening for IgM anti-CMV antibody may be practiced only for high risk recipients.

- Seronegative blood component is utmost essential for high risk patients. However, if seronegative components are not available, IgG anti-CMV seropositive leukoreduced components may be used.

- Due to dearth of seronegative donors in developing countries like India, latest techniques like pathogen inactivation may be made practically available in few centers catering high risk groups.

LIMITATIONS

- Follow up of the seronegative donors could have been done to look for any seroconversion

- Larger number of samples could have been studied

Sl. No	NAME	DONOR ID	AGE	SEX	BLOOD GROUP	MARITAL STATUS	OCCUPATION	EDUCATION	SOCIO ECONOMIC STATUS	TTI STATUS	IgM Anti-CMV	IgG Anti-CMV	PCR
1	Danesh kanth	2844	18	M	A1+	single	student	Graduate	middle class	NEG	Neg	Positive	
2	rukmangathan	2848	20	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
3	G Arun	2850	19	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
4	Rajesh	2853	22	M	B+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
5	pragadeeswari	2845	21	F	A1+	single	student	Graduate	middle class	NEG	Neg	Positive	
6	Thangarajan	2854	32	M	B+	Married	Clerical	Post Graduate	middle class	NEG	Neg	Positive	
7	Manikandan	2846	22	M	A1B+	single	student	Graduate	middle class	NEG	Neg	Positive	
8	saranraj	2849	20	M	O+	single	coolie	illiterate	low class	NEG	Neg	Positive	
9	Malarvizhi	2860	33	F	O+	Married	Clerical	higher secondary	middle class	NEG	Neg	Positive	
10	Sethubadmanaban	2856	19	M	B+	single	student	Graduate	middle class	NEG	Neg	negative	negative
11	Mahendra Prabu	2859	19	M	O Neg	single	coolie	illiterate	low class	NEG	Neg	Positive	
12	Balu	2843	28	M	O+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
13	Sujitha	2861	22	F	A1+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
14	Selvaraj	2847	33	M	O+	Married	coolie	illiterate	low class	NEG	Neg	Positive	
15	Balaji	2858	22	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
16	vasantha	2852	29	F	B+	Married	Professional	Post Graduate	middle class	NEG	Neg	Positive	
17	Ramanan	2857	38	M	A1+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
18	Naveena	2851	19	F	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
19	Sidharth	2842	19	M	A1+	single	student	Graduate	middle class	NEG	Neg	Positive	
20	Sathish Kumar	2855	19	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
21	LakshmiNarayanakumar	2863	38	M	O+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
22	Saravanakumar	2862	22	M	A1+	single	Clerical	Graduate	middle class	NEG	Neg	Positive	
23	Abdulwaheed	2865	34	M	O+	Married	Professional	Post Graduate	high class	NEG	Neg	Positive	
24	sabari	2864	20	F	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
25	Venkateswari	3453	22	F	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
26	kumaresan	3452	27	M	O+	Married	Professional	Post Graduate	high class	NEG	Neg	Positive	
27	Balu	3437	27	M	A1+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
28	Ramprathap	3449	25	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
29	Mannarsamy	3450	24	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
30	Nandhakumar	3438	25	M	A1+	single	Professional	Graduate	high class	NEG	Neg	negative	negative
31	Gopika	3454	22	F	A1B+	single	student	Graduate	middle class	NEG	Neg	Positive	
32	Palaniappan	3456	26	M	B+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	

Sl. No	NAME	DONOR ID	AGE	SEX	BLOOD GROUP	MARITAL STATUS	OCCUPATION	EDUCATION	SOCIO ECONOMIC STATUS	TTI STATUS	IgM Anti-CMV	IgG Anti-CMV	PCR
33	Nandakumar	3457	25	M	A1+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
34	Parthiban	3442	26	M	B+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
35	Balaji	3444	26	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
36	Sundar	3448	25	M	A Neg	single	Clerical	Graduate	middle class	NEG	Neg	Positive	
37	Ranjithkumar	3443	25	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
38	Kalaivani	3445	26	F	B+	Married	Professional	Graduate	high class	NEG	Neg	Positive	
39	Govardhanan	3435	25	M	O+	single	Clerical	Graduate	middle class	NEG	Neg	Positive	
40	Nareshkumari	3459	19	F	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
41	Dineshkumar	3446	24	M	O+	single	Professional	Graduate	middle class	NEG	Neg	negative	negative
42	Anandan	3441	26	M	O+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
43	Paneerselvam	3447	26	M	A1+	single	coolie	higher secondary	low class	NEG	Neg	Positive	
44	Deepika	3436	25	F	A1+	single	Professional	Graduate	high class	NEG	Neg	Positive	
45	Paramasivan	3458	34	M	A1+	Married	coolie	illiterate	low class	NEG	Neg	Positive	
46	Gangadaran	3455	25	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
47	Thangarathinam	3451	25	M	A1+	single	Clerical	secondary	low class	NEG	Neg	Positive	
48	Prabhu	3440	26	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
49	Shanmathi	3439	26	F	B Neg	Married	Professional	Graduate	high class	NEG	Neg	Positive	
50	Vivek	3153	18	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
51	Prakash	3168	19	M	O+	single	student	Graduate	high class	NEG	Neg	negative	negative
52	Neela	3147	18	F	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
53	rathinam	3167	20	F	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
54	vinotha	3165	19	F	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
55	Sivakumar	3152	19	M	A Neg	single	student	Graduate	middle class	NEG	Neg	Positive	
56	Praveenkumar	3150	19	M	A+	single	coolie	illiterate	low class	NEG	Neg	Positive	
57	selvakumari	3159	20	F	B+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
58	Alukmanharzeen	3166	21	M	A+	single	student	Graduate	middle class	NEG	Neg	negative	negative
59	Vinothkumar	3154	21	M	A Neg	single	student	Graduate	middle class	NEG	Neg	Positive	
60	Salimalik	3145	19	M	O+	single	coolie	illiterate	low class	NEG	Neg	Positive	
61	Venkatesh	3155	20	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
62	Kameshwaran	3148	19	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
63	Veerabhathiran	3160	32	M	A+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
64	Ratheesh	3149	23	F	A+	single	Professional	Post Graduate	high class	NEG	Neg	Positive	

Sl. No	NAME	DONOR ID	AGE	SEX	BLOOD GROUP	MARITAL STATUS	OCCUPATION	EDUCATION	SOCIO ECONOMIC STATUS	TTI STATUS	IgM Anti-CMV	IgG Anti-CMV	PCR
65	Anandan	3164	19	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
66	Dwarka	3144	20	F	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
67	Vinothkumar	3157	20	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
68	Jeevanandan	3151	21	M	O+	single	coolie	illiterate	low class	NEG	Neg	Positive	
69	Parthiban	3158	19	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
70	Jagan mohini	3146	18	F	O Neg	single	student	Graduate	middle class	HBsAg	Neg	Positive	
71	Vimalraj	3156	19	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
72	Saravanan	3169	18	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
73	Giriprashanth	3170	18	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
74	Rajkumari	2838	21	F	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
75	Madheshwaran	2822	23	M	A1+	single	Professional	Graduate	high class	NEG	Neg	Positive	
76	Karthika	2823	23	F	B+	single	Professional	Post Graduate	high class	NEG	Neg	Positive	
77	Vijaykrishnan	2833	30	M	B+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
78	Sathish Kumar	2825	26	M	A+	single	Professional	Graduate	middle class	NEG	Neg	negative	negative
79	LakshmiNarayanakumar	2821	26	F	O+	Married	Professional	Graduate	high class	NEG	Neg	Positive	
80	Bsikaisan	2828	20	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
81	Sheiksirajudeen	2824	22	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
82	Azhagarsamy	2834	29	M	A+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
83	Purishothaman	2832	20	M	B Neg	single	student	Graduate	middle class	NEG	Neg	Positive	
84	saravana Vidhya	2831	35	F	A2+	Married	coolie	illiterate	low class	NEG	Neg	Positive	
85	Rajesh	2835	22	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
86	Sadhamhussain	2840	20	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
87	Vajiravelu	2819	29	M	B+	Married	Professional	Graduate	high class	NEG	Neg	Positive	
88	Arunkumar	2841	27	M	O+	Married	Professional	Graduate	middle class	NEG	Neg	negative	negative
89	Meganathan	2827	21	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
90	Devendran	2830	20	M	AB+	single	student	Graduate	middle class	NEG	Neg	Positive	
91	Kumari	2826	19	F	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
92	Kavitha	2820	19	F	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
93	Ashokkumar	2836	24	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
94	Paulraj	2677	32	M	AB+	Married	coolie	illiterate	middle class	NEG	Neg	Positive	
95	Murthy	2698	40	M	AB+	Married	coolie	illiterate	low class	NEG	Neg	Positive	
96	Vaasantha	2693	22	F	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	

Sl. No	NAME	DONOR ID	AGE	SEX	BLOOD GROUP	MARITAL STATUS	OCCUPATION	EDUCATION	SOCIO ECONOMIC STATUS	TTI STATUS	IgM Anti-CMV	IgG Anti-CMV	PCR
97	Sundarajan	2676	36	M	AB+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
98	Jeevanandham	2674	23	M	B+	single	Clerical	secondary	low class	NEG	Neg	Positive	
99	Gilbertraj	2678	36	M	B+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
100	Sundarapandiyan	2680	25	M	A1+	single	Professional	Post Graduate	middle class	NEG	Neg	Positive	
101	Anumathon	2683	25	F	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
102	Kannusamy	2691	30	M	O+	Married	coolie	illiterate	low class	NEG	Neg	Positive	
103	Francis	2675	31	M	O+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
104	Rengaraja	2672	26	M	B+	single	Professional	Graduate	high class	NEG	Neg	negative	negative
105	Sathish Kumar	2742	23	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
106	Karunanidhi	2733	42	M	B+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
107	Narayani	2741	24	F	O+	single	Professional	Graduate	high class	NEG	Neg	Positive	
108	Nagraj	2711	25	M	A+	single	Clerical	higher secondary	low class	NEG	Neg	Positive	
109	sundaramurthy	2716	23	M	B+	single	Professional	Graduate	high class	NEG	Neg	Positive	
110	Pandian	2724	46	M	O+	Married	Clerical	secondary	middle class	NEG	Neg	Positive	
111	Silambarasan	2743	23	M	B Neg	single	student	Graduate	middle class	NEG	Neg	Positive	
112	Saravanan	2723	21	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
113	Jegadeeswari	2703	25	F	AB+	single	Professional	Graduate	high class	NEG	Neg	Positive	
114	Sathish	2715	25	M	B+	single	coolie	illiterate	low class	NEG	Neg	Positive	
115	sathiyasundaram	2705	22	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
116	Madhusudhanan	3363	18	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
117	Senthilnathan	3369	21	M	A+	single	student	Graduate	middle class	NEG	Neg	negative	negative
118	Vigneshwari	3361	19	F	A Neg	single	student	Graduate	middle class	NEG	Neg	Positive	
119	Sathish	3362	18	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
120	Raguram gayathri	3379	20	F	O+	single	coolie	illiterate	low class	NEG	Neg	Positive	
121	Ganapathy	3374	19	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
122	Mohammadarif	3383	19	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
123	Kumaran	3386	18	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
124	Abdulajees	3366	19	F	O+	single	coolie	illiterate	low class	NEG	Neg	Positive	
125	Raghupathy	3359	19	M	A1+	single	student	Graduate	middle class	NEG	Neg	negative	negative
126	ManoJequson	3364	20	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
127	Praveena	3360	19	F	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
128	Anburaj	3373	19	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	

Sl. No	NAME	DONOR ID	AGE	SEX	BLOOD GROUP	MARITAL STATUS	OCCUPATION	EDUCATION	SOCIO ECONOMIC STATUS	TTI STATUS	IgM Anti-CMV	IgG Anti-CMV	PCR
129	Ramalingam	3372	18	M	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
130	Prabakaran	3381	19	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
131	Surajeef	3389	18	F	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
132	Sourabh	3377	18	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
133	Sarathkumar	3375	20	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
134	Manojkumar	3365	18	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
135	Venkatesh	3378	20	M	B+	single	coolie	primary	low class	NEG	Neg	Positive	
136	Akhil	3368	19	F	A+	single	student	Graduate	middle class	NEG	Neg	Positive	
137	Sakthinathan	3367	19	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
138	Mohankumar	3376	19	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
139	Kevin	3370	18	M	AB+	single	student	Graduate	middle class	NEG	Neg	Positive	
140	Praveenkumar	3358	18	M	O+	single	student	Graduate	middle class	NEG	Neg	Positive	
141	Vidhya	2974	25	F	O+	Married	Clerical	primary	low class	NEG	Neg	Positive	
142	Jacob Antony	2975	27	M	O+	Married	Professional	Graduate	middle class	NEG	Neg	negative	negative
143	Manikandan	2986	32	M	B+	Married	coolie	high school	low class	NEG	Neg	Positive	
144	Banupradeep	2999	24	M	O Neg	single	Professional	Graduate	high class	NEG	Neg	Positive	
145	Radhika	2988	24	F	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
146	Pandian	2980	40	M	A1+	Married	Professional	Graduate	high class	NEG	Neg	Positive	
147	Ashokkumar	2993	26	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
148	Karthika	2991	28	F	A1+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
149	Shanmuganathan	2997	19	M	B+	single	student	Graduate	middle class	NEG	Neg	negative	negative
150	Miteshvarma	2990	25	M	A1+	single	Professional	Graduate	high class	NEG	Neg	Positive	
151	Anand	2979	34	M	A1+	Married	Professional	Post Graduate	middle class	NEG	Neg	Positive	
152	Altongo	2983	26	M	O+	single	coolie	primary	low class	NEG	Neg	Positive	
153	Thriveni	2996	23	F	A1+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
154	Ramasundarajan	3006	25	M	B+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
155	Venka	3014	24	F	A1+	single	Clerical	primary	low class	NEG	Neg	Positive	
156	Aravindan	3018	28	M	AB+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
157	Kirubashankar	3017	26	M	O Neg	single	Professional	Graduate	high class	NEG	Neg	Positive	
158	Balu	3015	28	M	B+	single	Clerical	higher secondary	middle class	NEG	Neg	Positive	
159	Naveen	3011	23	M	A1+	single	student	Graduate	middle class	NEG	Neg	Positive	
160	Arajitsarkar	3008	28	M	B+	Married	Professional	Graduate	high class	NEG	Neg	Positive	

Sl. No	NAME	DONOR ID	AGE	SEX	BLOOD GROUP	MARITAL STATUS	OCCUPATION	EDUCATION	SOCIO ECONOMIC STATUS	TTI STATUS	IgM Anti-CMV	IgG Anti-CMV	PCR
161	Madhanmohan	3004	33	M	B+	Married	Clerical	higher secondary	middle class	NEG	Neg	Positive	
162	Srivudhya	3012	24	F	B+	single	Professional	Graduate	high class	NEG	Neg	Positive	
163	Dhilipraj	3002	23	M	A1+	single	student	Graduate	middle class	NEG	Neg	Positive	
164	Chandrasekaran	3013	24	M	B+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
165	Kadharmoideen	3010	21	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
166	Boopalan	3016	25	M	O+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
167	Sriram	799	39	M	A+	Married	coolie	illiterate	low class	NEG	Neg	Positive	
168	Krishnakumari	801	35	F	O+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
169	Muthukumaran	803	23	M	A1B+	single	Professional	Post Graduate	middle class	NEG	Neg	Positive	
170	Zaheer Mohammad	822	30	M	O+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	
171	Praveen kumar	817	29	M	A1+	Married	coolie	illiterate	low class	NEG	Neg	Positive	
172	Rajamanickam	813	27	M	A1+	single	Professional	Graduate	middle class	NEG	Neg	positive	
173	Subhashini	811	27	F	O+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
174	Jafarali	807	23	M	B+	single	student	Graduate	middle class	NEG	Neg	Positive	
175	Sivakumar	805	28	M	A1+	single	coolie	illiterate	low class	NEG	Neg	Positive	
176	Vikramkumar	819	24	M	B+	single	Professional	Graduate	middle class	NEG	Neg	Positive	
177	Karthik	815	29	M	A2B+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
178	Sakthivel	827	32	M	O+	Married	Professional	Post Graduate	high class	NEG	Neg	negative	negative
179	Sivakumar	823	36	M	O+	Married	Professional	Graduate	middle class	NEG	Neg	Positive	
180	Raghuram	825	29	M	B+	Married	Clerical	Graduate	middle class	NEG	Neg	Positive	

The Tamilnadu Dr. M.G.R. Medical University

Department of Transfusion Medicine

Licence No. 191/28C

BLOOD DONOR FORM

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

Blood Bag No.	Date			Group & Rh

Personal Particulars

Donor's Name	Age :	Sex : Male / Female
Residence Address	**Office Address**	
.....	.....	
.....	.....	
..... Ph :	..... Ph :	

KIND ATTENTION

Kindly furnish the following information sought on medical grounds as per Government Notification. If any question is felt embarrassing kindly bear with us

TEMPORARY DEFERRAL, IN THE PAST 12 MONTHS HAVE YOU

- Received Tranfusion of Blood or its products Y/N
- Suffered from Hepatitis or had Hepatitis Immunoglobulin or had close contact with an individual suffering from Hepatitis Y/N
- Had exposure to tattoos, acupunture or body piercing? Y/N
- Had antirabies vaccine or was treated for dog bite? Y/N
- Undergone any major surgery or met with any major acciddent? Y/N

IN THE PAST 6 MONTHS HAVE YOU EVER

- Suffered from Typhoid / Cholera / Acute infection of kidney or Bladder Y/N
- Had delivery / had pregnancy / any abortion / or been breast feeding? Y/N / N/A*
- Had any major surgery or met with any minor accident? Y/N

* N/A - Not applicable

IN THE PAST 3 MONTHS

- Have you donated blood, plasma or platelets? Y/N
- Have yow been treated for malaria? Y/N
- Have you had any history of measles, mumps and chickenpox? Y/N

IN THE PAST 1 MONTH

- Had treatment for acne with Isotretinoin? Y/N
- Had Anti tetanus serum, Anti venom serum, Anti diphtheria serum, Anti gas gangrene serum or Rubella vaccination? Y/N

IN THE PAST 3 WEEKS

- Have you had tooth extraction or any dental procedure? Y/N

IN THE PAST 2 WEEKS

- Have you had chicken pox, shingles, measles, mumps or yellow fever vaccination Y/N

IN THE PAST 1 WEEK

- Have you had cortisone for treatment? Y/N
- Had history of diarrhea with fever? Y/N

IN THE PAST 4 DAYS

- Have you had IV antibiotics? Y/N

IN THE PAST 3 DAYS

- Have you had oral antibiotics? Y/N

IN THE PAST 24 HOURS

- Have you had alcoholic drinks Y/N
- Are you an aircrew, a heavy machine vehicle driver, a construciton worker? Y/N
- Are you reporting for duty in the next 12 hours? Y/N
- Are you suffering from cold, cough, sore throat or acute sinusitis? Y/N

PERMANENT DEFERRAL

H/o. Uncontrolled blood pressure or stroke?	Y/N
H/o. Heart disease or arrhythmias?	Y/N
H/o. Epilepsy or anticonvulsants?	Y/N
H/o. Auto immune disease or immounsuppressive therapy?	Y/N
H/o. Abnormal bleeding tendencies?	Y/N
H/o. Diabetic mellitus on treatment with insulin or hypoglycemic drugs?	Y/N
H/o. Chronic liver disease or endocrine disorders?	Y/N
H/o. Diabetic mellitus on treatment with insulin or hypoglycemic drugs?	Y/N
H/o. Chronic liver disease or endocrine disorders?	Y/N
H/o. Parkinsons diseases?	Y/N
H/o. Psorasis or treatment for the same?	Y/N
H/o. Psychiatric disorders?	Y/N
H/o. Major surgeries for kidney, heart, liver or brain?	Y/N
H/o. Severe allergic disorders or asthmatic on steroid therapy?	Y/N
H/o. IV drug abuse, heterosexual/homosexual promiscuity / STD?	Y/N

GENERAL QUESTIONS

1. Have you donated blood? Y/N
2. When was your last blood donation? .....

   How many times have you donated? .....
3. Are you willing to donate for emergency situations? Y/N
4. Have you had any reactions like giddiness/fainting attacks/ fits after donation? Y/N
5. Any history of unexplained weight loss/ chronic cough / fever / diarrhoea / Lymph nodes enlargement? Y/N

DECLARATION

I hereby declare that the above information is true to the best of my knowledge and this consent of mine to be a blood donor is voluntary. I understood that certain tests (HIV, HCV, HBV, SYPHILIS, MALARIA), will be performed on my blood for the purpose of ensuring the safety.

I would like to know the results, if any positive. Y/N

Date Signature of donor

PHYSICAL EXAMINATION

Wt (in Kg)	HB gms %	PR	BP	RR	TEMP.	CVS	RS	CNS	ABD	Skin disease at phlebotomy site

The above donor is FIT / UNFIT to donate blood.

Blood Bag : SINGLE / DOUBLE / TRIPLE

Volume : 350 ml /450 ml

Signature of the MEDICAL OFFICER.

LIST OF ABBREVIATIONS

AABB	-	American Association of Blood Banks
BMT	-	Bone Marrow Transplantation
CMV	-	Cytomegalovirus
ELISA	-	Enzyme Linked Immunosorbent Assay
FFP	-	Fresh Frozen Plasma
HbsAg	-	Hepatitis `B' surface antigen
HBV	-	Hepatitis B Virus
HCV	-	Hepatitis `C' Virus
HHV	-	Human herpes virus
HIV	-	Human Immunodeficiency Viruses
HTLV	-	Human T-cell lymphotropic Virus
IFN	-	Interferon
IL-6	-	Interleukin- 6
NAT	-	Nucleic acid Amplification Testing
PBMNC	-	Peripheral Blood Mononuclear Cells
RT-PCR	-	Real Time Polymerase Chain Reaction
TNF	-	Tumour Necrosis Factor
TT-CMV	-	Transfusion Transmitted Cytomegalovirus
TTI	-	Transfusion Transmitted Infections

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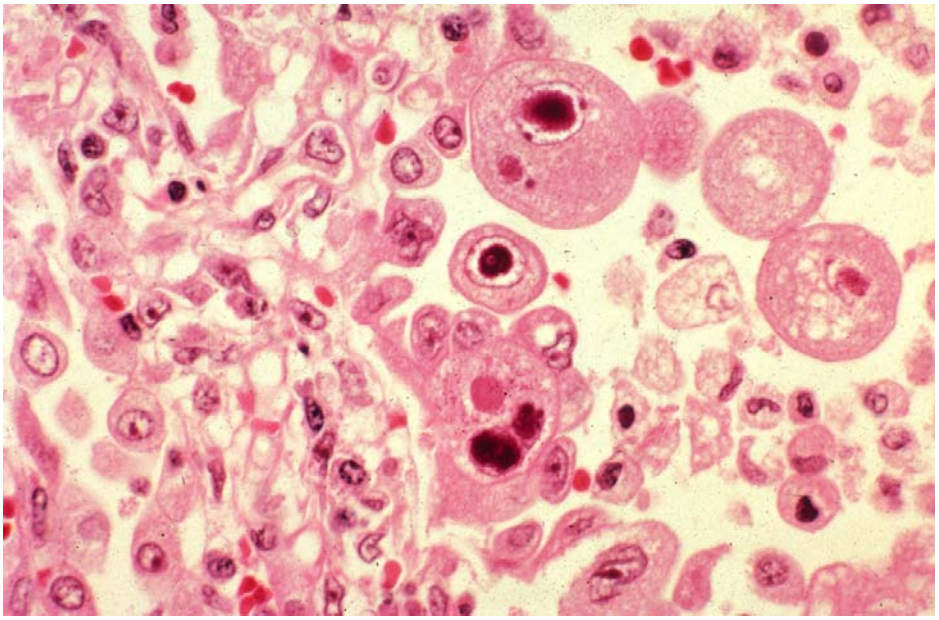


Fig 1. CMV infected cell showing cytomegalia with nuclear and cytoplasmic inclusions

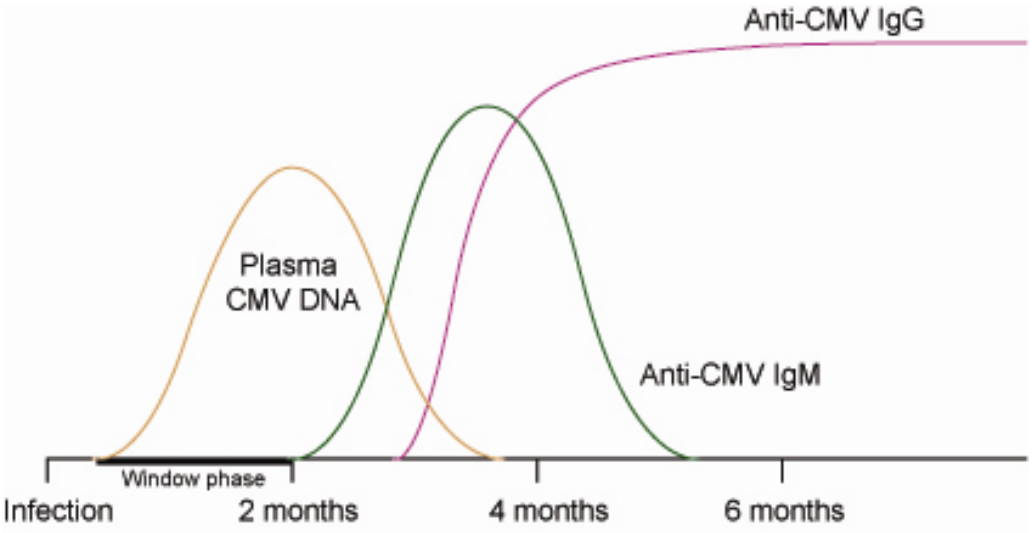


Fig 2. Immune response after primary CMV infection

Fig 3. CMV IgG ELISA Kit

Fig 4. CMV IgM ELISA Kit

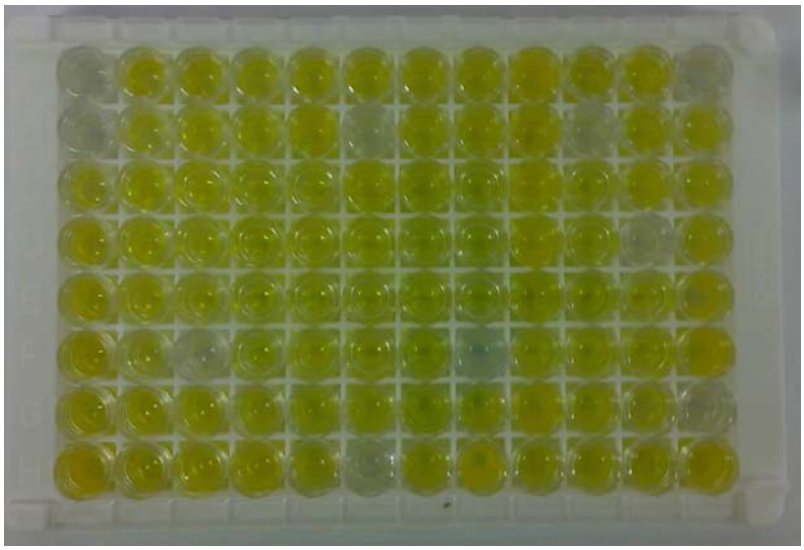


Fig 5. IgG anti-CMV ELISA

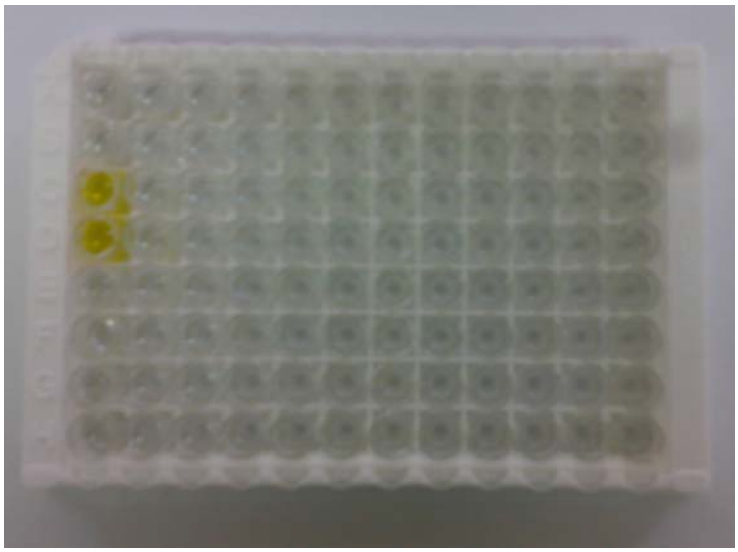
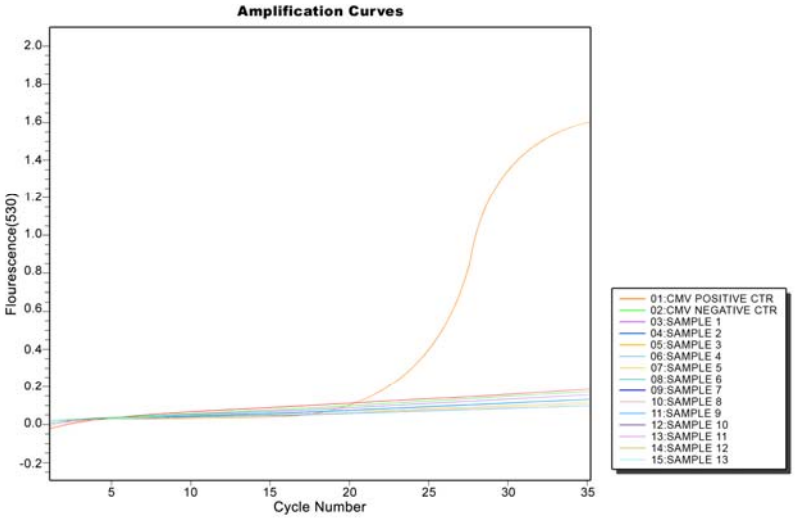
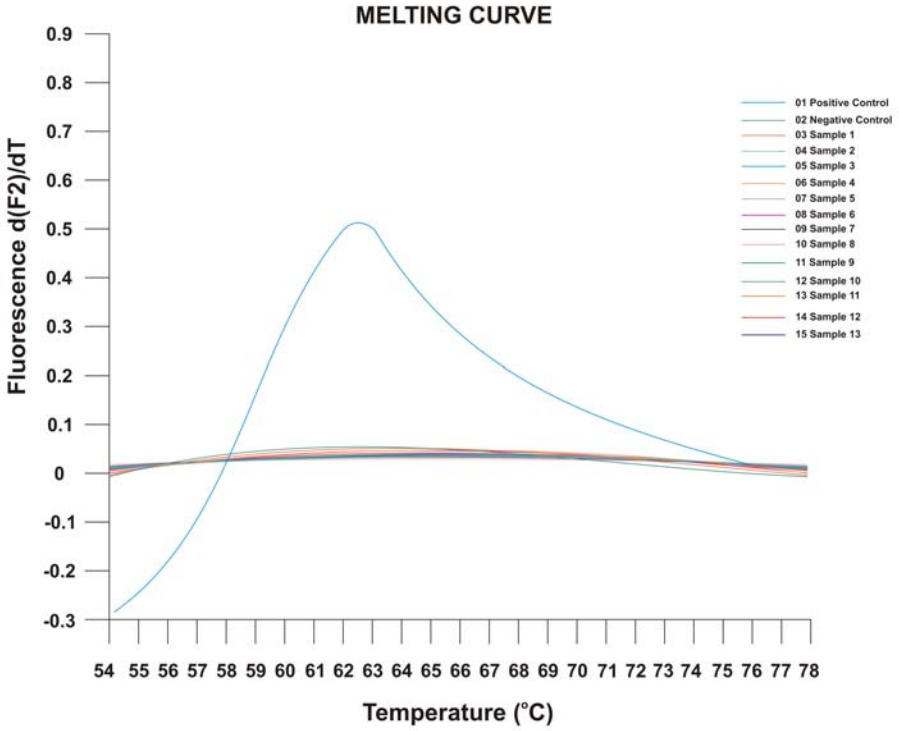


Fig 6. IgM anti-CMV ELISA

Fig 7. ROCHE Light Cycler

CMV DNA DETECTION BY RT - PCR




MELTING CURVE
Fluorescence d(F2)/dT
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78
Temperature (°C)
01 Positive Control
02 Negative Control
03 Sample 1
04 Sample 2
05 Sample 3
06 Sample 4
07 Sample 5
08 Sample 6
09 Sample 7
10 Sample 8
11 Sample 9
12 Sample 10
13 Sample 11
14 Sample 12
15 Sample 13